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The Effects of Glucosinolates and Their Hydrolysis Products on Cancer Types

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Abstract: Brassicaceae species, together with being a very important material for nutrition; chemically, anticarcinogenic, carcinogenic, antiestrogenic, estrogenic, antioxidant with antibiotic activity and inducing phase-II detoxification enzymes. Depending on the amount of these kinds of vegetables consumed by humans and animals, some ositive or negative metabolic effects of this molecule may occur. At the same time, liver, thyroid, kidney, stomach, colon, rectum abnormalities and development and reproductive performance can cause deterio-ration. ITCs inhibit mitosis and stimulate apoptosis in tumor cells.

Key Words: Glucosinolates, isothiosinolate, nitrile, cancer.

Glukosinolatlar ve Hidroliz Ürünlerinin Kanser Türleri Üzerine Etkileri

Özet: Brassicaceae türleri, beslenme için çok önemli bir materyal olmakla birlikte; kanserojenik, antiöstrojenik, östrojenik, antibiyotik aktivitesi olan antioksidan ve faz-II detoksifikasyon enzimlerini tetikleyen kimyasal maddeler içerir. İnsanlar ve hayvanlar tarafından tüketilen bu tür sebzelerin miktarına bağlı olarak, bu molekülün bazı olumlu veya olumsuz metabolik etkileri ortaya çıkabilir. Aynı zamanda karaciğer, tiroit, böbrek, mide, kolon, rektum anormallikleri ve gelişimi ve üreme performansının bozulmasına neden olabilir. İsothiosinolatlar, mitozu inhibe eder ve tümör hücrelerinde apoptozu uyarır.

Anahtar Kelimeler: Glucosinolatlar, isothiosinolat, nitril, kanser.

Introduction

The Structure of Glucosinoletes

Brassicaceae, is an economically important family for its many food and oilseed crops as well as containing many important ornamental plants and noxious weeds. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates. Several other plant families in the same plant order as the Brassicaceae have been found to possess glucosinolates. Glucosinolates are glucose and sulfur-containing organic anions (Fig. 1) whose decomposition products are produced when plant cells are ruptured, and the glucosinolates present in vacuoles are hydrolyzed by the enzyme myrosinase (-thioglucosidase glucohydrolase; EC 3.2.3.1)[1].

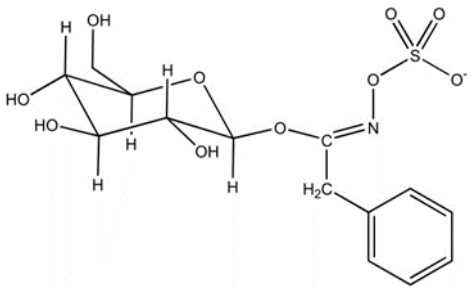

Figure 1. *The structure of glucosinoletes*
Şekil 1. *Glucosinolatların yapısı*

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Glucosinolates and Their Degradation Products

These hydrolysis products, many with biological activity, include substituted isothiocyanates (ITC), nitriles, thiocyanates, epithionitriles, and oxazolidinethiones, which vary depending on the plant species studied, side-chain substitution, cell pH, and cell iron concentration[2,3]. Some of these degradation products have been found to be potent phytotoxins. The leaves and seeds of garlic mustard have been previously shown to contain a high percentage of glucosinolates (up to 3% offresh weight in seeds), with the predominant glucosinolate being allyl glucosinolate (sinigrin)[4,5].

Many glucosinolate degradation products are of interest because of their biological activities. Several of these hydrolysis products have biocidal activity against a wide variety of organisms, such as insects, plants, fungi, and bacteria[6]. Others have human health benefits. For example, sulforaphane, a degradation product of the glucosinolate glucoraphanin, is a potent inducer of phase II detoxification enzymes, enzymes that are strongly correlated with the prevention of certain types of cancer[7]. Nutritional/health studies of these compounds require gram quantities, most of which are not commercially available. Certain other glucosinolate degradation products are utilized in organic synthesis, such as 3-methoxybenzyl isothiocyanate from meadow foam seed glucosinolates in the synthesis of substituted thioureas[8] (Fig. 2).

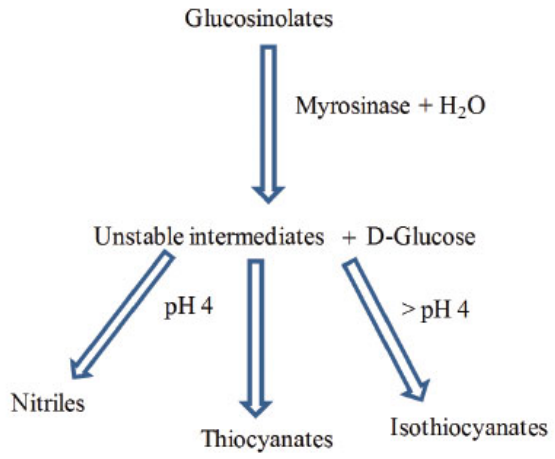

Figure 2. *Glucosinolates and their degradation products*

Şekil 2. *Glucosinolatlar ve yıkım ürünleri*

An overview of the epidemiological data concerning the cancer-preventive effect of brassica vegetables, including cabbages, kale, broccoli, Brussels sprouts, and cauliflower. A protective effect of brassicas against cancer may be plausible due to their relatively high content of glucosinolates. Certain hydrolysis products of glucosinolates have shown anticarcinogenic properties. The results of six cohort studies and 74 case-control studies on the association between brassica consumption and cancer risk are summarized. The cohort studies showed inverse associations between the consumption of brassica's and risk of lung cancer, stomach cancer, all cancers taken together. Of the case-control studies 64% showed an inverse association between consumption of one or more brassica vegetables and risk of cancer at various sites. Although the measured effects might have been distorted by various types of bias, it is concluded that a high consumption of brassica vegetables is associated with a decreased risk of cancer. This association appears to be most consistent for lung, stomach, colon and rectal cancer, and least consistent for prostatic, endometrial and ovarian cancer. It is not yet possible to resolve whether associations are to be attributed to brassica vegetables per se or to vegetables in general[9,10].

Prevalence of Brassicaceae Species and Relation with Cancer

Brassicaceae species, together with being a very important material for nutrition; chemically, anticarcinogenic, carcinogenic, antiestrogenic, estrogenic, antioxidant with antibiotic activity and inducing phase-II detoxification enzymes. At the same time, liver, thyroid, kidney, stomach, colon, rectum abnormalities and development and reproductive performance can cause deterioration. ITCs inhibit mitosis and stimulate apoptosis in tumor cells. In addition, ITCs are used as an alternative treatment for cancer treatment because they suppress the dephosphorylation of the C-Jun N-Terminal kinase. Brassica vegetables have protective effects against cancers particularly lung and digestive system related[11]. However, recent epidemiological studies have shown that; anti-cancer reactions are open to debate[12-14]. The positive relationship between Brassica vegetables and the reduction of cancer risk was determined by Van Poppel G. et al.[15] and Talalay and Fahey, 2001[16], Petri et al. 2003[17] and Hu et al. 2003[18] reported that there was no such relationship.

For example, Stoner et al. 2002[19], who studied indol-3-carbinol (I3C) and 3,3-diindolmethane (DIM), hydrolysis products of GLS, showed that I3C produced a 4-fold increase in liver of animals receiving carcinogen; Babich et al. 1993[20], Reddy et al. 1983[21] and Nishie and Daxenbichler 1980[22] reported that in humans I3C produced a 40% reduction in the surface of the cancerous colon. Petterson et al., 2002[11] have shown that such vegetables inhibit chemically colon cancer in experimental animals. It turns out that both GLS and hydrolysis products have both carcinogenic and anticarcinogenic effects. Because of these dual functions, the GLS are called "Janus Carcinogens"[23]. In addition, low doses of GLS and hydrolysis products activate protein kinases, while high doses activate cysteine proteases that cause apoptosis and potential cytotoxicity[24-26].

Brassicas, including all types of cabbages, broccoli, cauliflower and Brussels sprouts, may be protective against cancer due to their relatively high glucosinolate content. Some of the hydrolysis products, indoles and isothiocyanates, are able to influence phase 1 and phase 2 biotransformation enzyme activities, thereby possibly influencing several processes related to chemical carcinogenesis, e.g. the metabolism, DNA-binding and mutagenic activity of promutagens. A reducing effect on tumor formation has been shown in rats and mice. The anticarcinogenic action of isothiocyanates and indoles depends upon many factors, such as the test system, the target tissue, the type of carcinogen challenge and the anticarcinogenic compound, their dosage, as well as the timing of the treatment. Most evidence concerning anticarcinogenic effects of glucosinolate hydrolysis products and brassica vegetables has come from studies in animals. Animal studies are invaluable in identifying and testing potential anticarcinogens. In addition, studies carried out in humans using high but still realistic human consumption levels of indoles and brassica vegetables have shown putative positive effects on health[27].

The protective effect of brassicas against cancer may be due to their relatively high content of glucosinolates. Certain hydrolysis products of glucosinolates have shown anticarcinogenic properties. The results of 7 cohort studies and 87 case-control studies on the association between brassica consumption and cancer risk are summarized. The cohort studies showed inverse associations between the consumption of cabbage, cauliflower, and broccoli and risk of lung cancer; between the consumption of brassicas and risk of stomach cancer; between broccoli consumption and risk of all cancers taken together; and between brassica consumption and the occurrence of second primary cancers. Of the case-control studies, 67% showed an inverse association between consumption of total brassica vegetables and risk of cancer at various sites. For cabbage, broccoli, cauliflower, and Brussels sprouts, these percentages were 70, 56, 67, and 29%, respectively. Although the measured effects might have been distorted by various types of bias, it is concluded that a high consumption of brassica vegetables is associated with a decreased risk of cancer. This association appears to be most consistent for lung, stomach, colon, and rectal cancer and least consistent for prostatic, endometrial, and ovarian cancer. It is not yet possible to resolve whether associations are to be attributed to brassica vegetables per se or to vegetables in general. Further epidemiological research should separate the anticarcinogenic effect of brassica vegetables from the effect of vegetables in general[28].

Over several decades a number of epidemiological studies have identified the inverse associations between cruciferous vegetables and the risk of several cancers, including gastric, breast, colo-rectal, lung, prostate, bladder and endometrial cancers, via plausible physiological mechanisms. Although retrospective case-control studies have consistently reported inverse associations between the risk of these cancers and the intake of cruciferous vegetables and isothiocyanate-containing plants, current prospective cohort studies have found these associations to be weaker and less consistent. Genetic variations affecting the metabolism of glucosinolate hydrolysis products may modulate the effects of cruciferous vegetable consumption on cancer risk, which may be one of the reasons for the discrepancies between retrospective and prospective studies. In addition, methodological issues such as measurement errors of dietary exposure, misclassification, recall bias, publication bias, confounding and study design should be carefully considered in interpreting the results of case-control and cohort studies and in drawing conclusions in relation to the potential effects of cruciferous vegetables on cancers. Although recent comprehensive reviews of numerous studies have purported to show the specific protective role of cruciferous vegetables, and particularly Brassicas, against cancer risk,

the current epidemiological evidence suggests that cruciferous vegetable consumption may reduce the risk only of gastric and lung cancers. However, there is at present no conclusive evidence that the consumption of cruciferous vegetables attenuates the risk of all other cancers[29].

Epidemiological studies give evidence that cruciferous vegetables (CF) protect humans against cancer, and also results from animal experiments show that they reduce chemically induced tumor formation. These properties have been attributed to alterations in the metabolism of carcinogens by breakdown products of glucosinolates, which are constituents of CF. The present article gives an overview on the present state of knowledge on the impact of CF and their constituents on enzymes that are involved in the metabolism of DNA-reactive carcinogens. The development of in vitro models with metabolically competent cell lines led to the detection of potent enzyme inducers contained in CF such as sulforaphane. Recently, we showed that Brassica juices induce glutathione-S-transferases (GST) and cytochrome P-450 1A2 in human hepatoma cells (HepG2) and protect against the genotoxic effects of B(a)P and other carcinogens. Earlier in vivo experiments with rodents indicated that indoles and isothiocyanates, two major groups of glucosinolate breakdown products, attenuate the effects of polycyclic aromatic hydrocarbons (PAHs) and nitrosamines via induction of GST and inhibition of cytochrome-P450 isoenzymes, respectively. Our own investigations showed that CF are also protective towards heterocyclic amines (HAs): Brussels sprouts- and garden cress juices attenuated IQ-induced DNA-damage and preneoplastic lesions in colon and liver of rats. These effects were paralleled by induction of uridine-di-phospho-glucuronosyl transferase (UDPGT) which is very probably the mechanism of protection against HAs by cruciferous vegetables. There is also evidence that consumption of CF might protect humans against cancer. In matched control intervention studies with these vegetables, it was shown that they induce GST-activities in humans but overall, results were inconclusive. Recently, we carried out crossover intervention studies and found pronounced GST-induction upon consumption of Brussels sprouts and red cabbage, whereas no effects were seen with white cabbage and broccoli. Furthermore, we found that the isoenzyme induced was GST-pi which plays an important role in protection against breast, bladder, colon and testicular cancer. No induction of the GST-alpha isoform could be detected. Urinary mutagenicity experiments gave further evidence that CF affect drug metabolism in humans. Consumption of red cabbage led to changes in the pattern of meat-derived urinary mutagenicity. Overall, CF are among the most promising chemopreventive dietary constituents and further elucidation of their protective mechanisms and the identification of active constituents may contribute to the development of highly protective Brassica varieties[30].

Epidemiological data indicate that consumption of cruciferous vegetables is associated with a decreased incidence of cancer in human populations[31]. The anti-carcinogenic properties of cruciferous vegetables and isolated compounds have been studied in several investigations. When animals were first fed on diets high in cruciferous vegetables and then exposed to various indirect-acting carcinogens, tumour yields were lower and survival rates were higher than similarly-treated animals on semi-purified diets. A number of these cruciferous plants, including Brussels sprouts, cabbage, cauliflower and broccoli have been found to induce aryl hydrocarbon hydroxylase (EC 1.14.14.1) activity and/or the activity of cytosolic glutathione-S-transferase (EC 2.5.1.18)[32,33].

Colorectal cancer (CRC) is ranked as the fourth most com-mon cancer worldwide with approximately 944 000 casesbeing diagnosed in 2000, accounting for 9.2 % of all newcancer cases. It is the second most common cause of death from malignant neoplasms in the EU, with 190000 new cases per year. The cancer occurs almost equally inmen and women, as demonstrated in wester nised countries, where CRC represents 12.6 % of all incident cancers in menand 14.1% in women. The majority of epidemiological studies evaluating theassociation between fruit and vegetable consumption andcolon cancer risk have repor ted inverse associations although some recent studies have reported conflictingresults. The association between vegetables and colon cancerappears to be stronger for the dark green vegetables and among the subgroups of these vegetables, Brassica vegetables have shown strong negative associations betweenconsumption and colon cancer risk in both sexes.

Measuring oxidative DNA damage in human lymphocytes, in addition to antioxidant status in the blood cells, gives anidea of the

integrated rate of DNA damage in the body andis suggested to be a potential biomarker for cancer risk. Consumption of Brussels sprouts, spinach, watercress, ora sprouting vegetable mixture (containing broccoli, radish, alfalfa and clover) significantly reduced DNA damage inlymphocytes, following treatment (ex vivo) with H_2O_2, andas measured via 8-oxod G excretion. A good cor-relation has been observed between DNA damage occur-ring in colonocytes and the levels observed in lymphocytesof subjects participating in supplementation studies. Therefore effects observed in peripheral lymphocytesshould be consistent with site-specific effects, such as those seen in the colon[34].

Conclusion

Further epidemiological research should separate the anticarcinogenic effect of brassica vegetables from the effect of vegetables in general. The mechanisms by which brassica vegetables might decrease the risk of cancer are reviewed in this paper. Most evidence concerning anticarcinogenic effects of glucosinolate hydrolysis products and brassica vegetables has come from studies in animals. In addition, studies carried out in humans using high but still realistic human consumption levels of indoles and brassica vegetables have shown putative positive effects on health. The combination of epidemiological and experimental data provide suggestive evidence for a cancer preventive effect of a high intake of brassica vegetables.

The main biological effects observed in animals after expo-sure to Brassica vegetables or purified ITCs are changes inenzyme activities, decreased levels of DNA damage andreductions in colonic aberrant crypt foci (ACF) formation. ACF, thought to be the earliest morphological changes tooccur during colonic mucosal neoplasia have been observed in the human colon and in rats and mice treated with carci-nogens, and have been used as a surrogate marker for colon cancer for assessing activity of chemoprotective agents.

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