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Chemopreventive mechanisms and other properties of glucosinolates*

Mechanizmy chemoprewencyjne i inne właściwości glukozynolanów

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ABSTRACT
The increasing incidence of cancer is driving research into new methods of cancer prevention. Chemoprevention is the use of natural or synthetic substances to slow down, stop or delay carcinogenesis. Increased intake of cruciferous plants may have a beneficial effect on reducing the incidence of human cancers. This is related to the presence of glucosinolates, i.e. biologically active substances whose enzymatic degradation products have a detoxifying effect, inhibiting neoplastic processes and adhesion, and intensifying tumour cell apoptosis.
Keywords: glucosinolates; chemoprevention; health-promoting properties; adverse effects.

ABSTRAKT
Rosnąca zachorowalność na choroby nowotworowe skłania do poszukiwania nowych metod zapobiegawczych. Chemoprewencja to wykorzystywanie substancji pochodzenia naturalnego lub syntetycznego w celu spowolnienia, zahamowania lub opóźnienia kancerogenezy. Zwiększone spożycie roślin krzyżowych może wpływać korzystnie na zmniejszenie występowania nowotworów u ludzi. Jest to związane z obecnością w tych roślinach glukozynolanów, czyli substancji biologicznie czynnych, których produkty enzymatycznego rozpadu wykazują działanie detoksykacyjne, hamujące procesy nowotworzenia, adhezje oraz nasilające apoptozę komórek nowotworowych.
Słowa kluczowe: glukozynolany; chemoprewencja; właściwości prozdrowotne; działania niepożądane.

INTRODUCTION

The World Health Organization is warning that cancer diseases are second only to circulatory diseases as the most common cause of death in Europe. In 2012, some 14 million new cases were reported. The number of cancer sufferers is anticipated to keep growing in the coming years, reaching 19 million in 2025 [1]. Such a global health situation drives the search for new prophylactic measures, including chemoprevention [2].

It was demonstrated that biologically active substances found in cruciferous plants (*Cruciferae* family) of the genus Brassica may protect against developing cancer. Properties inhibiting carcinogenesis are associated with the presence of glucosinolates (GLS) in those plants [3]. Glucosinolates are organic compounds found in the plants from the families *Brassicaceae, Capparaceae* and *Caricaceae*, such as cabbage (white, red, savoy and Chinese), broccoli, cauliflower, Brussels sprouts, kale, common radish, rocket, horseradish, garden cress or rapeseed. Glucosinolates are a group of more than 200 compounds which are different in terms of composition and structural properties, including anthocyanins, thiocyanates, nitriles and indole. They are characterised by high stability and resistance to high temperatures. Apart from chemopreventive properties, GLS display antibacterial, antifungal and antioxidant activity [4].

CARCINOGENESIS AND CHEMOPREVENTIVE MECHANISMS

Carcinogenesis is a complex multi-stage process, which is usually initiated by exposure of the cells to a carcinogen, a cancer-causing agent. Stage one – initiation – is characterised by genotoxic alterations, whereby healthy cells are transformed into cancer cells. The subsequent promotion stage involves epigenetic alterations affecting gene expression through DNA methylation and histone modifications [5]. The final stage of carcinogenesis – progression – is characterised by spontaneous cell proliferation and elaboration of angiogenesis factors [5], expression of genes encoding proteolytic enzymes and adhesion alterations in cell membrane transporters [6].

Measures aimed at counteracting carcinogenesis at every stage of cancer formation, fall under the umbrella term of chemoprevention [6]. The concept of chemoprevention was introduced in the 1970s and refers to the use of biologically active substances for the prevention, suppression or reversal of cancer progression [7].

Three main chemopreventive processes have been identified:
- primary chemoprevention (anti-initiation),
- secondary chemoprevention (anti-promotion),
- tertiary chemoprevention.

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Anti-initiation chemoprevention inhibits tumour formation in the initial stage of carcinogenesis. It is dedicated to the healthy population and groups with a genetically-conditioned increased risk of cancer [2]. At this stage, the role of GLS is to prevent the formation of neoplastic lesions by reducing the activity of enzymes promoting carcinogenesis, stimulating enzymes involved in detoxifying processes, the so-called phase I and II enzymes, neutralizing reactive oxygen species (ROS) and activating DNA repair pathways [6, 8]. The next step is secondary chemoprevention, which comprises anti-promotion and anti-progression activities [2, 6]. Its goal is to halt tumour growth and assist in the treatment of identified premalignant lesions [2]. Glucosinolates and their degradation products were found to inhibit oncogene activity, slow down the proliferation of cancer cells, induce apoptosis, and also to downregulate the inflammatory response and angiogenesis [6, 8]. Tertiary chemoprevention is targeted to prevent recurrence and second primary cancers [2]. It also plays a part in maintaining the therapeutic effect and supporting chemotherapy [6].

CHEMOPREVENTIVE MECHANISMS OF GLUCOSINOLATES

The use of GLS of plant origin may be beneficial in preventing cancer diseases. Importantly, their properties are most effective at the early stages of carcinogenesis. Numerous studies demonstrate that including vegetables like cabbage, broccoli, Brussels sprouts or cauliflower into the daily diet reduces the risk of developing cancers, including pancreatic [9], breast [10], prostate [8], colon [11, 12] and liver cancer. It was also demonstrated in *in vitro* studies that the seed hydrolysate of *Lobularia libyca*, belonging to the Brassicaceae family, had an inhibitory effect on the development of colorectal, hepatic and breast cancer cell lines [4]. The mechanism of chemopreventive activity of sulforaphane (SFN) glucosinolate is presented in Figure 1.

Modulation of phase I and II detoxification – primary chemoprevention

Glucosinolates and products of their metabolism exert a stimulating effect on the body's defences during carcinogenesis. They were found to modulate the activity of phase I and II detoxification enzymes. Generally speaking, phase I detoxification primarily involves the inhibition of enzymes involved in activating xenobiotics, while phase II improves the solubility of a carcinogen or another toxic compound and effectively removes it from the body [6, 13]. Phase I detoxification enzymes catalyse reactions of oxidation, reduction, hydrolysis and dehalogenation, and generate functional groups for phase II detoxification, when their water solubility is increased and they are eliminated from the body [6].

Phenethyl isothiocyanate (PEITC), SFN, indole-3-carbinol and 3,3'-diindolylmethane (DIM), which are all GLS, are capable of modulating the enzymes of phase I (inhibiting cytochrome P450) and phase II detoxification, regulating transcription factors, signalling pathways, phases of the cell cycle and cancer cell apoptosis [9].

Isothiocyanates have an inhibitory effect on cytochrome P450 phase I enzymes which facilitate binding of carcinogens to DNA and cause mutations [6]. Sulforaphane, a compound within the isothiocyanate group, exerts anti-proliferative action on cancer cells and upregulates their apoptosis and differentiation [13]. Additionally, SFN was observed to have a suppressive effect on CYP1A and CYP2B1/2 in studies on rat hepatocytes [13]. Sulforaphane and other isothiocyanates activate such phase II detoxification enzymes as: glutathione S-transferases (GST), UDP-glucuronosyltransferases (UGT) and quinone oxidoreductases, e.g. NQO1 [6]. Reactions catalysed by those enzymes make xenobiotics more hydrophilic and easier to excrete from the body [13]. Studies on cell line and animal models demonstrated a positive correlation between the administration of SFN and higher mRNA levels of phase II detoxification enzymes, such as UGT1A1, GSTA1, NQO1 UGT1A1 in hepatocytes; UGT1A1 and

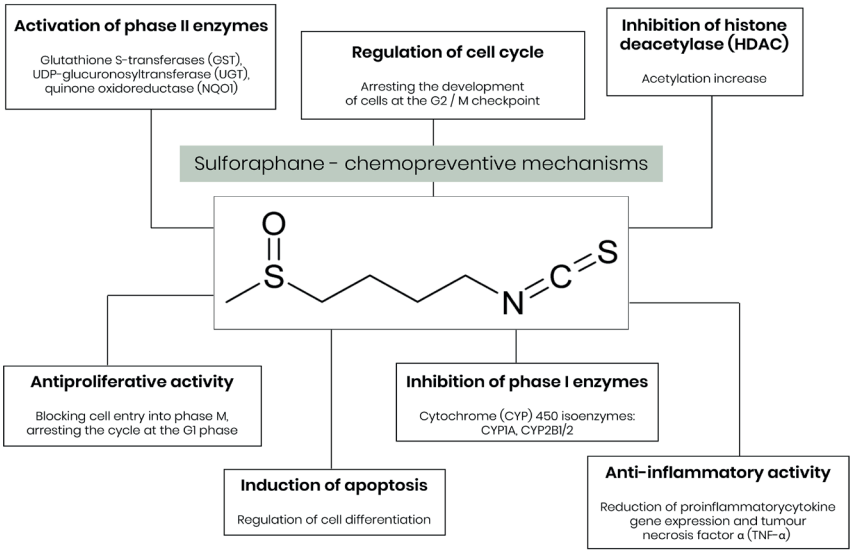

FIGURE 1. Chemopreventive activity of glucosinolates, illustrated by sulforaphane

GSTA1 in human colorectal cells; NQO1 in prostate cells; NQO1 and GST in the liver, kidneys and urinary bladder; as well as GSTA1 and UGTA1 in enterocytes [13].

Regulation of cell cycle

Glucosinolates and their degradation products exert a chemopreventive action also at the carcinogenesis stages of promotion and progression, destroying cancer cells. When DNA damage occurs, the cell cycle may be arrested for repair, but serious or irreversible damage triggers the apoptotic pathway [6]. The cell cycle may be arrested at checkpoints corresponding to the G1, S, G2 and M phases. Cell line studies demonstrated that SFN regulates the cell cycle, arresting the development of cancer cells at the G2/M checkpoint [8, 13]. Additionally, it was reported that SFN blocks cancer cells from entering the M phase, thus inhibiting their proliferation. Suppression of the development of colorectal and prostate cancer cells has been demonstrated. Other studies showed the inhibitory effects of SFN on those cells by arresting the cycle at the G1 phase [13]. However, the capability of inducing apoptosis or inhibiting proliferation is associated with the quantity of SFN used and length of exposure [14]. Short-term exposure led to a reversible cell cycle arrest in colorectal cancer cells at the G2/M checkpoint, while irreversible cell arrest and induction of apoptosis required exposure in excess of 12 h [15].

Induction of apoptosis

The process of apoptosis makes it possible to eliminate cells that are damaged, redundant or genetically modified, like cancer cells. However, during proliferation, cancer cells lose the ability to respond to apoptotic signals [6]. *In vitro* studies demonstrated that SFN intake induced apoptosis in colorectal, prostate, breast, liver, pancreas and brain cancer cells and in leukaemia cells [13, 16]. Apoptosis was also induced in hepatocellular carcinoma cells HepG2 by SFN exposure lasting 48 h [17].

Anti-inflammatory activity

The anti-inflammatory properties of GLS and their degradation products are associated with blocking transcription factor NF-κB (nuclear factor kappa B) which has a procarcinogenic effect. Nuclear factor kappa B regulates the expression of genes involved in the proliferation and growth of cancer cells, their invasiveness, angiogenesis and apoptosis. Inflammatory responses enhance cell proliferation and inhibit apoptosis, thus increasing the risk of cancer [6, 18]. Nuclear factor kappa B forms an inactive complex with inhibitor of κB (IκB) which is retained in the cytoplasm. Upon the influence of external factors, i.e. stress or pathogens, IκB is deactivated and consequently NF-κB is free to enter the nucleus, initiating mechanisms responsible for the production of proinflammatory cytokines [6]. By blocking NF-κB, isothiocyanates downregulate the secretion of pro-inflammatory signalling molecules which are released by nucleated blood cells [6]. In an animal study, macrophages were exposed to lipopolysaccharide (LPS) to induce inflammation. The administration of isothiocyanates (allyl-isothiocyanate and SFN) reduced the gene expression of inducible nitric oxide synthase (iNOS) and proinflammatory cytokines: interleukin 1 (IL-1β) and tumour necrosis factor (TNF-α) [19]. Downregulation of proinflammatory factors was linked with the effect of isothiocyanate analogs on NF-κB. Inhibitory effects were demonstrated for DIM, PEITC and SFN in the transcription of NF-κB, and consequently that of proinflammatory mediators, such as interleukin 6 (IL-6), iNOS, TNF-α and cyclooxygenase 2 (COX-2) [20].

Epigenetic modulation

The internal homeostasis of the cell, and particularly of the nucleus, is an important aspect of chemoprevention. Deoxyribonucleic acid bound to proteins (histones) undergoes various modifications, and maintaining the balance from one reaction to the next has implications for the body as a whole, e.g. through the processes of acetylation and deacetylation. Histone acetyltransferases (HATs) stimulate the binding of DNA to transcription factors, which makes histone acetylation possible. Opposite effects are produced by histone deacetylases (HDACs), which reduce the accessibility of DNA to transcription factors. In normal cells, HATs and HDACs remain in homeostasis, while in cancer cells their balance is impaired [6, 18]. It was demonstrated that a decline in the histone acetylation state contributes to cancer development and its subsequent recurrence. Histone deacetylases are over-expressed in cancer cells. Studies have shown that HDAC inhibitors, e.g. suberoylanilide hydroxamic acid (SAHA), valproic acid, depsipeptide and sodium butyrate, are effective against cancer, notably prostate cancer, by inhibiting growth and inducing apoptosis [18]. The administration of SFN to a prostate cell culture model led to HDAC inhibition and an increase in acetylation of histone H3 at the p21 promoter gene, as well as increased acetylation of alpha-tubulin leading to cell death [18].

In a study on mice given a single oral dose of SFN at 10 μmol, a significant reduction in HDAC activity was observed in the mucosa of the colon and inhibition of tumour development in mice with APC gene mutation, causing multiple intestinal neoplasia [18]. Histone deacetylase inhibitor activity is also demonstrated by PEITC, which acts by reducing the transcriptional and post-transcriptional expression of androgen receptor (AR). This is related to increasing the activity of the transcription factor Sp1 – which regulates AR expression in the development of prostate cancer. Phenethyl isothiocyanate also improves the expression of glutathione S-transferase Pi 1 (GSTP1) – phase II detoxification enzyme, which is suppressed in prostate cancer cells [18, 21].

Effect of glucosinolates on oestrogen-dependent cancers

Breast cancer and cervical cancer are regarded as oestrogen-dependent conditions. Indole-3-carbinol was demonstrated to have a modifying effect on oestrogen structure, suppressing the proliferation of cancer cells. Additionally, under the low-pH conditions of the stomach the compound is transformed into DIM, which in turn upregulates oestrogen metabolism, reducing blood oestrogen levels [10]. Binding of indole-3-carbinol and DIM to the aryl hydrocarbon receptor (AhR) slows down

oestrogen-dependent cancer cell proliferation. Activation of AhR upregulates the expression of CYP1A1, CYP1A2, ALDH1A3 and ALDH3A1, and consequently contributes to lowering oestrogen levels and accelerating its metabolic rate [22].

ANTIBACTERIAL AND ANTIFUNGAL ACTION

Helicobacter pylori infection is a common problem affecting many populations. The risk of infection is increased for people who do not have access to clean, safe food and water [23]. A positive correlation was found between *Helicobacter pylori* infections and subsequent incidence of duodenal and gastric ulcer disease as well as an elevated risk of stomach cancer [20].

One study found that SFN isolated from broccoli seeds added to isolated colonies of *H. pylori* had a bacteriostatic effect on 3 reference strains and 45 clinical isolates. At MIC90 (minimal inhibitory concentration at which growth of 90% of strains is inhibited) of ≤4μg/mL, the bactericidal activity of SFN was higher than that of other substances of plant origin, namely resveratrol (MIC90 = 25 μg/mL), allixin (MIC90 = 25 μg/mL), protolichesterinic acid (MIC90 = 32 μg/mL) or epigallocatechin gallate (MIC90 = 32 μg/mL) [24]. It was demonstrated that seed extracts of *Lobularia libyca*, containing glucoiberin, glucoerucin and glucoiberverin, have a suppressive effect against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.*

BIOCIDAL ACTION

With the growing level of environmental awareness, there is a tendency to replace synthetic pesticides with substances of natural origin in pest management. Biofumigation employs biocidal compounds occurring naturally in plants. An *in vitro* study revealed that extracts of various *Brassica* species containing GLS as well as glucosinolate hydrolysis products inhibited the development of plant pathogen colonies – bacteria: *Xanthomonas campestris* and *Pseudomonas syringa* and fungi: *Alternaria brassicae* and *Sclerotinia scletoriorum* [25]. Biocidal properties are exhibited by thiocyanates, nitriles, epithionitriles and isothiocyanates which are hydrolysis products of GLS. Isothiocyanates derived from the degradation of gluconasturtiin and glucoraphanin demonstrated biocidal effects against Gram-negative bacteria, such as *Agrobacterium tumefaciens, Erwinia chrysanthemi, Pseudomonas cichorii, Pseudomonas tomato, Xanthomonas junglandis*, which cause yellowing and darkening of leaves, and the development of rot in fruit, stems and roots [26]. Allyl-isothiocyanate, a degradation product of sinigrin, exhibits bactericidal properties against strains of the *Salmonella* genus and species of *E. coli* [26].

ADVERSE EFFECTS OF GLUCOSINOLATES

Irrespective of the broad spectrum of beneficial effects attributed to GLS, an excessive consumption may have some adverse effects. One of the most common downsides of GLS is their goitrogenic action. Excessive consumption of cruciferous plants may suppress thyroid secretion activity, resulting in depressed blood levels of thyroid hormones: triiodothyronine (T3) and tetraiodothyronine (T4). The pituitary gland responds to the low levels of T3 and T4 by increasing the production of the thyroid-stimulating hormone, which stimulates the thyroid to grow in size and weight, producing a goitre. The phenomenon depends on the iodine content in the body, and the risk of developing goitre is lower when adequate amounts of iodine are supplied in the diet.

Animal studies demonstrated that sustained administration of indole-3-carbinol after the impact of a carcinogenic factor stimulates tumour growth. The procarcinogenic effect was particularly pronounced with liver, thyroid and uterine cancers. Nevertheless, the current body of knowledge is not sufficient to permit a precise determination of the adverse effects of indole-3-carbinol and its condensation products on human cancer development [6].

CONCLUSIONS

Glucosinolates have a broad spectrum of activity, including inhibition of growth of some bacteria, like *Helicobacter pylori*, *Staphylococcus aureus, Pseudomonas aeruginosa*, as well as fungi, e.g. *Candida albicans*. The most important health benefits of those substances, however, result from their chemoprotective properties. They reduce the risk of developing cancer of the pancreas, breast, prostate, colon and liver. They are abundant in cruciferous vegetables, and therefore should be an essential part of the daily diet for adults and children. Apart from human health benefits, GLS may contribute to environmental protection by serving as natural plant protection agents. Glucosinolates are also attributed with some adverse effects, related to an excessive intake, but these require a more thorough analysis and research on a broader scale.

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