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In vitro assessment of antiproliferative action selectivity of dietary isothiocyanates for tumor *versus* normal human cells

In vitro ispitivanje selektivnosti antiproliferativnog dejstva dijetetskih izotiocijanata na tumorske u odnosu na normalne humane ćelije

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Abstract

Background/Aim. Numerous epidemiological studies have shown beneficial effects of cruciferous vegetables consumption in cancer chemoprevention. Biologically active compounds of different *Brassicaceae* species with antitumor potential are isothiocyanates, present in the form of their precursors – glucosinolates. The aim of this study was to determine the selectivity of antiproliferative action of dietary isothiocyanates for malignant *versus* normal cells. **Methods.** Antiproliferative activity of three isothiocyanates abundant in human diet: sulforaphane, benzyl isothiocyanate (BITC) and phenylethyl isothiocyanate, on human cervix carcinoma cell line – HeLa, melanoma cell line – Fem-x, and colon cancer cell line – LS 174, and on peripheral blood mononuclear cells (PBMC), with or without mitogen, were determined by MTT colorimetric assay 72 h after their continuous action. **Results.** All investigated isothiocyanates inhibited the proliferation of HeLa, Fem-x and LS 174 cells. On all cell lines treated, BITC was the most potent inhibitor of cell proliferation with half-maximum inhibitory concentration (IC_{50}) values of 5.04 mmoL m^{-3} on HeLa cells, 2.76 mmol m^{-3} on Fem-x, and 14.30 mmol m^{-3} on LS 174 cells. Antiproliferative effects on human PBMC were with higher IC_{50} than on malignant cells. Indexes of selectivity, calculated as a ratio between IC_{50} values obtained on PBMC and malignant cells, were between 1.12 and 16.57, with the highest values obtained for the action of BITC on melanoma Fem-x cells. **Conclusion.** Based on its antiproliferative effects on malignant cells, as well as the selectivity of the action to malignant *vs* normal cells, benzyl isothiocyanate can be considered as a promising candidate in cancer chemoprevention. In general, the safety of investigated compounds, in addition to their antitumor potential, should be considered as an important criterion in cancer chemoprevention. Screening of selectivity is a plausible approach to the evaluation of safety of both natural isothiocyanates and synthesised analogues of these bioactive compounds.

Key words:
isothiocyanates; vegetables; neoplastic cells, circulating; lymphocytes; chemoprevention.

Apstrakt

Uvod/Cilj. Brojne epidemiološke studije pokazale su povoljne efekte konzumiranja povrća iz familije kupusnjača (*Brassicaceae*) u hemioprevenciji karcinoma. Osnovni biološki aktivni sastojci ovog povrća su izotiocijanati, prisutni u obliku prekursora – glukozinolata. Cilj ovog rada bio je određivanje selektivnosti antiproliferativnog delovanja dijetetskih izotiocijanata na maligne ćelije u odnosu na normalne ćelije. **Metode.** Antiproliferativna aktivnost tri izotiocijanata zastupljena u ljudskoj ishrani: sulforafana (SFN), benzil-izotiocijanata (BITC) i feniletil-izotiocijanata (FEITC) na humane maligne ćelijske linije, HeLa, ćelijsku liniju karcinoma grlića materice, Fem-x, ćelijsku liniju melanoma, i LS 174, ćelijsku liniju karcinoma kolona, kao i na mononuklearne ćelije periferne krvi (MNĆPK), sa ili bez delovanja mitogena, određivana je MTT kalorimetrijskim testom, 72 h nakon kontinuiranog delovanja agenasa. **Rezultati.** Svi ispitivani izotiocijanati inhibirali su proliferaciju HeLa, Fem-x i LS 174 ćelija. Na svim ćelijskim linijama BITC je pokazao najizraženije delovanje sa vrednostima polumaksimalne inhibitorne koncentracije (IC_{50}) od 5.04 mmol m^{-3} na HeLa ćelijama, 2,76 mmoL m^{-3} na Fem-x i 14,30 mmol m^{-3} na LS 174 ćelijama. Svi ispitivani izotiocijanati pokazali su citotoksično delovanje na MNĆPK, ali sa višim IC50 vrednostima u odnosu na maligne ćelije. Indeksi selektivnosti antitumorkog delovanja, izraženi kao odnos IC_{50} vrednosti dobijenih na MNĆPK i malignim

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ćelijama, bili su između 1,12 i 16,57, sa najvišom vrednosti pri delovanju BITC na Fem-x ćelije. **Zaključak.** Na osnovu antiproliferativne aktivnosti na maligne ćelije i selektivnosti antiproliferativnog delovanja na maligne u odnosu na normalne ćelije, benzil izotiocijanat se ističe kao perspektivni agens u hemioprevenciji karcinoma. Generalno, pored antitumorskog delovanja, bezbednost primene ovih jedinjenja treba da predstavlja važan kriterijum u izboru odgovarajućih izotiocijanata za primenu u primarnoj, sekundarnoj i tercijarnoj hemioprevenciji kancera. Ispitivanje selektivnosti predstavlja pogodan pristup oceni bezbednosti i prirodnih izotiocijanata i sintetskih analoga.

Ključne reči:
izotiocijanati; povrće; neoplazme, cirkulišuće ćelije; limfociti; hemoprevencija.

Introduction

Numerous epidemiological studies have shown an inverse association between cruciferous vegetable intake and risk of different types of cancer [1–5]. Cruciferous vegetables, including broccoli, cauliflower, cabbage, kale, Brussels sprouts, water cress, and rocket, among others, are rich sources of sulphur containing compounds – glucosinolates (GLs). Basic structure of GLs consists of a β-D-thioglucose group, a sulfonated oxime group and a side chain derived from different amino acids. The most abundant source of these plant constituents is the family *Brassicaceae* (*Cruciferae)* in which they were discovered [6]. Glucosinolates undergo hydrolysis by endogenous enzyme myrosinase, to yield glucose, sulphuric acid and a molecule of thiocyanates, nitriles, indoles or isothiocyanates (ITCs) depending on the side chain in parent glucosinolate [7]. Myrosinase accompanies the GLs in the plant tissue, placed in cells separately from GLs and released only after the degradation of cell walls (by chopping, chewing), thus catalysing the generation of isotiocyanates. Intestinal flora also possesses myrosinase activity [7].

Numerous studies, both *in vitro* and *in vivo*, have indicated that the beneficial effects of cruciferous vegetables in cancer prevention are the result of the action of ITCs rather than the action of their precursors – glucosinolates [8, 9]. Mechanisms of anticancer activity of ITCs are numerous [10]. They act both as blocking and suppressing agents able to impede initiation, promotion and/or progression of carcinogenesis [11]. Inhibition of carcinogenesis during the initiation stage is presumably associated with the modulation of carcinogen metabolism, including inhibition of metabolic activation of carcinogens by phase I enzymes, coupled with induction of detoxifying phase II enzymes [12]. Inhibition of tumor cell proliferation is crucial for the inhibitory effects of ITCs on promotion and progression of carcinogenesis [13]. Other chemopreventive mechanisms include induction of apoptosis, prevention of neoangiogenesis, anti-migratory or epigenetic effects [8, 14]. Scientific evidence of antitumor potential of ITCs provides a rationale for their use as chemopreventive agents [15, 16]. Structural differences are the result of different side group in a relatively simple chemical structure of ITCs and determine specific mechanistic profile of individual molecules regarding both antitumor action and their safety [17]. Selectivity of ITCs action towards malignant cells is the main criterion for their potential use in primary, secondary or tertiary prevention. In general, the balance between efficacy and safety (in addition to the costs and practicality of the intervention) is crucial criterion in the evaluation of chemopreventive agents regardless the nature and mechanism of their action. The balance is shifted to the safety for agents with the main role in primary and secondary prevention, while in tertiary prevention and therapy efficacy takes the priority [18].

Numerous data have shown that even subtle change in chemical structure of the ITCs can have a profound effect on their activity and mechanism of action, which have opened a wide field of chemical synthesis of ITC analogues with targeted effects and increased efficacy [19–23]. Evaluation of their chemopreventive potential in terms of fine balance between efficacy and safety is an important issue for their further use in practice.

Sulforaphane (SFN), benzyl isothiocyanate (BITC) and phenyl ethyl isothiocyanate (PEITC) are ITCs often present in human diet (based on the intake of food rich in their precursor GLs). Cruciferous vegetables usually contain a large number of structurally different GLs. However, SFN, in the form of corresponding GL - glucoraphanin, is the major bioactive compound in broccoli, BITC in the form of glucotropaeolin, is the major bioactive in garden cress, and PEITC in the form of GL – gluconasturtiin, is major bioactive of watercress [24].

The aim of this study was to provide data on antiproliferative action of SFN, BITC and PEITC on a panel of malignant cell lines in the presence of human sera as a model closer to in *vivo* conditions and with specific focus on the selectivity of their antitumor action. Selectivity of antiproliferative action towards malignant cells was determined by simultaneous investigation of their effects on peripheral blood mononuclear cells (PBMC) of healthy volunteers. Applied experiments are proposed to be used as a plausible and practical screening model in the preclinical evaluation of cancer chemopreventive profile of natural isothiocyanates and synthesised analogues of these bioactive compounds, including both safety and efficacy.

Methods

Cell cultures and chemicals

Human cervix carcinoma cell line (HeLa), human melanoma cell line (Fem-x) and human colon cancer cell line (LS 174) used in the study were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell lines were cultured as monolayers in the nutrient medium, i.e. RPMI 1640 medium (Sigma–Aldrich, Germany) supplemented with L-glutamine (3 moL m^{-3}), streptomycin (100 mg L^{-1}), penicillin (100 IU mL^{-1}) and grown at 37°C in atmosphere with 5% CO_2, 95% air and 95% relative humidity.

Stock solutions of ITCs (Sigma–Aldrich, Germany) were made in dimetyl sulfoxide (DMSO) at concentrations of 28.3 moL

m^{-3} for SFN, 75.4 moL m^{-3} for BITC and 66.8 moL m^{-3} for PEITC, filtered through Milipore filter 0.22 μm and kept at – 20°C.

The whole blood for isolation of sera and PBMC was obtained from healthy subjects according to the protocol approved by Ethical Committee of the Institute for Oncology and Radiology of Serbia (within the project No 175011). The study was undertaken according to the Helsinki Declaration and all the subjects gave written informed consent prior to the enrolment. Human AB+ sera isolated from the whole blood of healthy volunteers was pooled and used further according to the protocol.

Preparation of peripheral blood mononuclear cells

PBMC were separated from the whole heparinized blood of healthy volunteers by gradient centrifugation (Lymphoprep™, Norway). Cells collected from the interface were washed three times with Haemaccel® (aqueous solution supplemented with 145 mol m^{-3} Na^+, 5.1 moL m^{-3} K^+, 6.2 moL m^{-3} Ca^{2+}, 145 moL m^{-3} Cl^- and 35 g L^{-1} gelatin polymers, pH = 7.4), counted and re-suspended in nutrient medium.

Treatment of cell lines

HeLa and Fem-X cells were seeded at a density of 2,000 cells *per* well in 96-well plates, in nutrient medium supplemented with human serum (a mass fraction of 10%) in total volume of 100 μL. LS 174 cells were set up at a density of 7,000 cells *per* well and grown similarly.

The following day, after the adherence, cells were treated with ITCs. Briefly, stock solutions were diluted in nutrient medium supplemented with fresh AB+ human serum (a mass fraction of 10%) and 50 μL of the obtained working solutions was added to the wells. Final concentrations of ITCs tested for antiproliferative action on cancer cell lines were 1, 5, 10, 25, 50 μM. Concentration of DMSO in the cell did not exceed 0.17%. Wells with cells treated with DMSO solution in nutrient medium, in concentrations equal to the DMSO level in wells with 50 μM ITCs (i.e. 0.17% in experiments with SFN, 0.07% with PEITC and 0.075 % with BITC) were used as controls. It was shown in preliminary experiments that in these concentrations DMSO do not influence the proliferation and the survival of the malignant or normal cells. Wells containing ITCs in investigated concentrations but void of cells were used as corresponding blanks.

Treatment of peripheral blood mononuclear cells

PBMC were seeded in 96-well plates (150,000 cells *per* well) in nutrient medium supplemented with 10% autologous human serum, or in supplemented nutrient medium enriched with phytohaemaglutinin (PHA; 5 mg L^{-1}; Sigma-Aldrich, Germany). Stock solutions of ITCs were diluted in nutrient medium supplemented with autologous serum (a mass fraction of 10%) and working solutions was added to the wells. Final concentrations of ITCs tested for their action on PBMC were 5, 10, 20, 40 and 80 μM. Wells with PBMC and DMSO solution in nutrient medium (up to 0.17%) were used as controls and wells containing ITCs in investigated concentrations but void of cells were used as corresponding blanks. The ranges of ITCs concentrations tested (0–50 μM for experiments on cancer cell lines and 0–80 μM on PBMC) were defined based on the preliminary experiments with the highest concentration selected as the one that induce the decrease in cell survival to the 10% of the cell number (i.e. absorbance) in the control wells.

Determination of cell survival

Cell survival was assayed 72 h after the continuous ITC action, using MTT test [25]. Briefly, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Sigma-Aldrich, Germany solution (5 μg L^{-1} in phosphate buffered saline; pH 7,2) was added to each well. Samples were then incubated for four hours at 37°C in 5% CO_2 and humidified air atmosphere. Afterwards, 100 μL of 10% sodium dodecyl sulfate was added to the wells and the plates were kept overnight in the CO_2 incubator in humidified atmosphere, followed by absorbance measurements. The absorbance (A) was measured at 570 nm. The cell survival (%) was calculated by division of A of sample with cells grown in the presence of various concentrations of investigated compounds, with control absorbance (Ac) of cells grown only in nutrient medium, and multiplied with 100. It was implied that A of blank was always subtracted from A of corresponding sample with target cells. The half-maximal inhibitory concentration (IC_{50}) was determined as a concentration of a compound that inhibited cell proliferation by 50% compared to control wells; i.e. that resulted in cell survival of 50%.

Statistical analysis

All results were presented as mean ± standard deviation (SD) of five independent experiments. All experiments are performed in triplicates. IC_{50} values were extracted from dose-response curves for each ITC on each cell type, as a concentration of a drug that inhibited cell survival by 50%.

Results

The results obtained for antiproliferative action of investigated compounds show that all investigated ITCs, SFN, BITC, and PEITC, significantly inhibited proliferation of cultured HeLa, Fem-x and LS 174 cells. Based on IC_{50} values (Table 1) BITC was the most potent inhibitor of cell proliferation in all cell lines treated. The potencies of SFN and PEITC were similar. The order of sensitivity of various human cancer cell lines to the antiproliferative action of ITCs in descending order was: human melanoma, Fem-x cells > human cervix adenocarcinoma, HeLa cells > human colon carcinoma, LS 174 cells.

The obtained results show that BITC has the most potent antiproliferative action compared to SFN and PEITC in HeLa and LS 174 cells. Our data from this work also show antiproliferative potency of tested ITC also on melanoma Fem–

Table 1

Half-maximal inhibitory concentration (IC_{50}) for the antiproliferative action of the investigated compounds on malignant and normal human cells determined by MTT assay after 72 h of continuous action

Cell line/compound	IC_{50} (mmoL m^{-3})		
	SFN	BITC	PEITC
HeLa	13.59 ± 1.53	5.04 ± 1.73	12.00 ± 1.97
Fem-x	6.67 ± 0.73	2.76 ± 0.58	6.22 ± 0.32
LS 174	16.09 ± 2.44	14.30 ± 5.16	18.23 ± 3.04
PBMC	29.34 ± 15.3	45.74 ± 18.9	23.41 ± 7.13
PBMC+PHA	15.30 ± 5.39	21.92 ± 4.63	30.87 ± 6.33

Data are presented as mean ± standard deviations (SD) of five independent experiments. SFN – sulforaphane; BITC – benzyl isothiocyanate; PEITC – phenyl ethyl isothiocyanate; PBMC – peripheral blood mononuclear cells; PBMC+PHA – PBMC treated with phytohaemagglutinin; HeLa – human cervix carcinoma cell line; Fem-x – human melanoma cell line; LS-174 – human colon cancer cell line; MTT – 3-(4, 5-dimethylthiazol-2-yl) – 2,5 diphenyltetrazolium bromide.

x cell line. It should be noted that this cell line appears to be the most sensitive one. IC_{50} values for the action of SFN and PEITC on Fem-x cells were similar. It should be emphasized that both values are similar to the IC_{50} value of (5.51 ± 0.3 mmoL m^{-3}) for the action of cisplatin on Fem-x cells that was reported by Pantelić et al. [26] in the same experimental design. Even stronger antiproliferative potential of BITC on Fem-x cells compared to the action of cisplatin, with twice as lower IC_{50} value was demonstrated in the present study.

As seen in Figure 1, the shape of dose-response curves for the action of ITCs on malignant cells and PBMCs is different. A characteristic plateau could be observed in the cur-

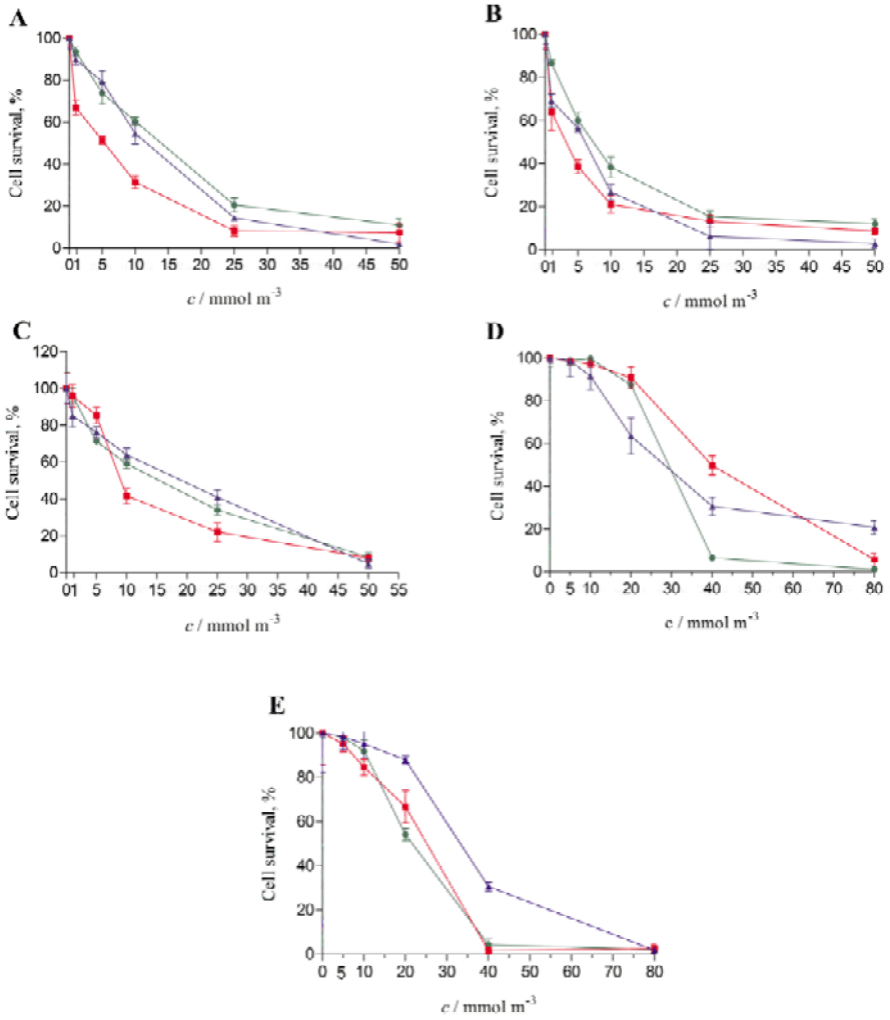

Fig. 1 – Representative graphs showing the survival of HeLa (A), Fem-x (B), LS 174 cells (C), peripheral blood mononuclear cells (PBMC, D) and PBMC stimulated with phytohaemagglutinin (E), plotted as a function of concentration (c) of SFN (●), BITC (■), and PEITC (▲), determined by MTT test 72 h after the continuous agents action. For other abbrevations see under Table 1.

ve for ITCs treated PBMC and PBMC with mitogen in the concentration range 0–5 mmoL m^{-3}, and a linear dose-response correlation with higher doses. Regarding the effects on malignant cells, linearity in the response could be observed within the whole concentration range.

The selectivity in the antitumor action was evaluated for each ITCs and was expressed as selectivity index (SI), calculated as the ratio between IC_{50} values obtained with PBMC or mitogen-treated PBMC and IC_{50} values for investigated ITCs on malignant cells. The values obtained for SI are presented in Table 2.

Table 2

Selectivity indexes (SI) of antiproliferative action of the investigated isothiocyanates

Cell lines/compound	SI*		
	SFN	BITC	PEITC
IC_{50} PBMC / IC_{50} HeLa	2.16	9.07	1.95
IC_{50} PBMC+PHA / IC_{50} HeLa	1.12	4.35	2.57
IC_{50} PBMC / IC_{50} Fem-x	4.39	16.57	3.76
IC_{50} PBMC+PHA / IC_{50} Fem-x	2.28	7.94	4.96
IC_{50} PBMC / IC_{50} LS 174	2.16	3.19	1.28
IC_{50} PBMC+PHA / IC_{50} LS 174	1.12	1.53	1.69

***SI towards malignant cell line are calculated as the ratio of the half-maximal inhibitory concentration (IC_{50}) value for PBMC (or PBMC+PHA) and IC_{50} value for corresponding cell line.**

SFN – sulforaphane; BITC-benzyl isothiocyanate; PEITC – phenyl ethyl isothiocyanate; PBMC – peripheral blood mononuclear cells; PBMC+PHA – PBMC treated with phytohaemagglutinin; HeLa – human cervix carcinoma cell line; Fem-x – human melanoma cell line; LS-174 – human colon cancer cell line.

Discussion

Investigation of the antiproliferative action of selected ITCs reported was performed in the presence of human sera (10%), including pooled AB positive sera from healthy donors used in experiments with malignant cells in culture and autologous sera for investigation of antiproliferative effects of ITCs on PBMC. As far as authors are aware the antiproliferative screening of ITCs in the presence of human sera was not performed previously. However, it has been shown that the presence of human umbilical blood sera has stimulatory influence on growth and proliferative capacity of human mesenchymal stem cells, without influence on their morphological and functional characteristics [27]. It has been shown also that both normal and neoplastic cells of nervous origin, cultured in the presence of human sera, have distinct adhesive characteristics, proliferation capacity and antigen expression compared to the same type of cells grown in the presence of calf serum [28]. Accordingly, the proposed experimental design that includes the presence of human sera with putative influence on phenotypic and functional characteristic of biological models could be considered as better approximation of *in vivo* conditions. However, obtained results are in accordance with the previously published data on the antiproliferative action of SFN, BITC and PEITC in HeLa, [29–31] as well as in LS 174 cells [32].

Additionally, cell models used for the selectivity assessment usually include normal cells of the same origin in addition to malignant cells [26] or PBMCs, as proposed in our work. Prioritisation of PBMCs as a model was rationalised by important role of PBMC subpopulations in the immunological control of tumor growth [33] and a possibility to include mitogen stimulated cells as a model of normal cells in the highly proliferative state.

PBMC from healthy individuals, stimulated for proliferation with PHA were similarly sensitive to the cytotoxic action of SFN as low sensitive HeLa and LS 174 cells. This is in consent with published data which show that after 72 h of incubation with 30 mmol m^{-3} of SFN only 16% of T-lymphocytes stimulated with PHA were viable compared to the control [34]. It is also in accordance with the recently published data that the action of BITC is particularly expressed on highly proliferative cells [35].

The obtained results showing lower IC_{50} values for BITC and SFN on PBMC treated with mitogen compared to untreated PBMC are in line with previously published data [14]. Action of PEITC characterised with lower IC_{50} values obtained in PBMC than in PBMC treated with mitogen, suggests different mechanism that needs to be investigated further with putative effects on both malignant and non-malignant lymphoproliferative diseases. Lower IC_{50} values on PBMC compared to cancer cells was most pronounced for BITC as observed also based on the selectivity indexes obtained that were in a wide range between 3.19 for LS 174 cells and 16.57 for Fem-x cells. This is a new result which marks BITC as promising cancer suppressive ITC with the highest selectivity index among the investigated compounds.

The observed differences in the antiproliferative potential of the investigated ITCs could be at least in part due to the differences in kinetics of cellular accumulation. Substantial accumulation within a cell is a general characteristics of the most of the investigated ITCs. However, the kinetics and the level of accumulation strongly depend on the nature of particular ITCs, incubation temperature and glutathione levels in the cell [36]. It was shown on Hepa 1clc7 liver cells that BITC accumulation is a fast process with maximum concentrations reached 30 minutes after the exposure, followed by rapid decline in intracellular concentration leading to complete clearance after 24 h [36]. Contrary to BITC, very slow intracellular accumulation was observed in cells exposed to the same concentrations of SFN with maximum levels reached 12 h after the exposure, followed by the slow decline with half of maximum levels detected at 24 h. This was confirmed in other cell lines treated with the same concentrations of bi-

oactives during 30 minutes. Surprisingly, intracellular levels of BITC were up to 3 times higher compared to the levels of SFN [36]. The capacity of ITCs to induce antioxidant enzymes activity has shown the inverse correlation with the accumulation kinetics and ITCs levels observed 24 h after the exposure, with SFN highlighted as the best inducer of their activity. Contrary to the influence on antioxidant enzymes activity, kinetics of the intracellular transfer was shown to be in direct correlation with the antiproliferative capacities of different ITCs. In a panel of malignant cells of different origin (HL60S, 8662/S, MCF-7, HepG2, HT-29, HaCaT) IC_{50} values obtained after 72 h of continuous exposure to BITC and PEITC were similar to the IC_{50} values obtained after the same period, i.e. 72 h that combines 3 h of direct exposure to ITCs followed by washings and subsequent 69 h long incubation without ITCs [36]. The results obtained for SFN were different in IC_{50} values after 72 h of exposure to SFN 10 times lower compared to the 3h-long exposure followed by 69 h of incubation without ITCs [36]. However, after short exposure to the same concentrations of ITCs, BITC accumulation in cells, levels of reactive oxygen species and antiproliferative capacity are much higher than in SFN treated cells [37]. The observed differences, mostly due to different side chains influencing their lipophilicity have major effects on intracellular action of ITCs. Within a cell all ITCs react directly *via* carbon atom of the –N=C=S group with the cysteine sulfhydryl groups of glutathione (GSH) and proteins. The side chains generally play secondary role, mainly by influencing the electrophilicity of the –N=C=S group and steric effects [38]. With greater ITCs influx and subsequent GSH depletion, cells are more sensitive to the effects of intracellular reactive oxygen species (ROS). On the contrary, slower influx and consequent slow decrease on GSH level could act as a signal for glutathione-S-transferase activation, resulting in higher GSH levels [38]. Malignant phenotype is characterised by high levels of oxidative stress, mainly due to high levels of ROS and disturbed ratio between reduced and oxidised form of GSH [39]. The persistent oxidative stress in cancer cells sensitizes them to stress or apoptotic effects of anticancer drugs, which often generate ROS, because they are already near a threshold for tolerating ROS. It is not the case with normal cells, with lower production of H_2O_2 [40]. It seems that the fate of cancer cells to survive the effects of ITC depends on constitutive levels of ROS and/or glutathione, the type, dose and accumulation kinetics of ITC the cells were exposed to, resulting in the difference in sensitivity of different cells types and the difference in ITCs selectivity regarding their antiproliferative action.

It has been shown previously that chemopreventive action of SFN, as the strongest inhibitor of phase I enzymes and inductor of phase II enzymes in this group of bioactives, is mediated mainly by the modulation of carcinogen metabolism [15]. Accordingly, SFN is the major ITC highlighted for the use in primary prevention at low dietary relevant doses, *via* its major dietary sources, including broccoli. However, the selectivity indexes for the action of SFN are lower compared to other investigated ITCs suggesting that SFN at higher doses (as isolated molecule or in enriched extracts) could affect proliferation of immunocompetent cells. It should be noted that the obtained results rationalize further investigation of putative beneficial effects of SFN on the suppression of limphoproliferative or autoimmune diseases.

Compared to SFN and PEITC, BITC has shown better characteristics as chemopreventive agent acting on the proliferation of cancer cells. The pronounced selectivity in antitumor action of this compound, by comparison of its effects on malignant cells and PBMC, favors this particular ITC for the use as cancer suppressive chemopreventive agent. BITC is a major ITC of garden cress, and it is not present in broccoli or in many other dietary sources of glucosinolates and isothiocyanates [24]. Thus, in addition to its beneficial effects the distribution in dietary plants should be taken into account in evidence-based personalised nutrition recommendation and the use of dietary sources of BITC in secondary or tertiary chemoprevention. Other dietary sources of glucotropeolin and BITC that are not part of westernised diet should be promoted to provide higher intake of this bioactive compound in subjects with high cancer risk or cancer patients.

Conclusion

Numerous biological effects of ITCs suggest their active role in dietary prevention of malignant and other chronic diseases that represent major burden to health worldwide. Our data contribute to the rationale for future comprehensive studies that could eventually lead to more specific dietary guidance and recommendations for increased intake of vegetables contaiining particular glucosinolates and ITCs, targeted to the specific populations such are cancer patients or subjects at high cancer risk. Further research and conclusions based on human intervention trials are needed to provide additional scientific evidence for the beneficial effects of long-term intake of BITC or its dietary sources in cancer patients, as agent in tertiary chemoprevention or even as a complementary therapeutic. ITCs are also considered as a good starting point for synthesis of functional analogues that will enhance their biological activity. However, the critical assessment of their safety should be included in the evaluation of their potential use in primary, secondary and tertiary prevention of cancer, as a part of balanced diet, functional component of functional foods and dietary supplements, chemopreventive agents and therapeutics. Screening of selectivity by applying a model presented in this work is a plausible approach for the preclinical evaluation of safety of both natural ITCs and synthesised analogues of these bioactive compounds.

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