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 čelije

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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 antigenetic 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 (IC50) 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 IC50 than on malignant cells. Indexes of selectivity, calculated as a ratio between IC50 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 versus 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 synthetized analogues of these bioactive compounds.

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

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LS 174, čelijsku liniju karcinoma kolona, kao i na mononukle- arne čelije perifernje krvi (MNČPK), sa ili bez delovanja mito- gena, određena je MTT kalorimetrijskim testom, 72 h nakon kontinuiranog delovanja agenasa. Rezultati. Svi ispitivani izo- tiocijanati inhibirali su proliferaciju HeLa, Fem-x i LS 174 čelija. Na svim čelijskim linijama BITC je pokazao najizraženije delovanje sa vrednostima polumaksimalne inhibitorne koncen- tracije (IC50) od 5.04 mmol m−3 na HeLa čelijskim linijama, 2.76 mmol m−3 na Fem-x i 14.30 mmol m−3 na LS 174 čelijskim linijama. Svi ispitivi- vani izotiocijanati pokazali su hitoksično delovanje na MNČPK, ali sa višim IC50 vrednostima u odnosu na maligne čelije. Indeks selektivnosti antitumorog delovanja, izraženi kao odnos IC50 vrednosti dobijeni na MNČPK i malignim
乙烯基化合物的活性对于恶性细胞的选择性和抗肿瘤活性是至关重要的。在检测恶性细胞的敏感性时，基质的选择是作为参考性参数的。一般来说，考虑到治疗的安全性（包括成本和实用性）以及对检测的研究，有必要在初步、中期和终期预防中研究其安全性。总的来说，平衡是在安全性的改变下，对于安全的性质和机制对于它们的化学预防剂而言是重要的。

Introduction

Numerous epidemiological studies have shown an inverse association between cruciferous vegetable intake and risk of different types of cancer. 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. Glucosinolates undergo hydrolysis by endogenous enzyme myrosinase, to yield sulphuric, sulphuric acid and a molecule of thiocyanate, nitriles, indoles or isothiocyanates (ITCs) depending on the side chain in parent glucosinolates. 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 isothiocyanates. Intestinal flora also possesses myrosinase activity.

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. Mechanisms of anticancer activity of ITCs are numerous. They act both as blocking and suppressing agents able to impede initiation, promotion and/or progression of carcinogenesis. 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. Inhibition of tumor cell proliferation is crucial for the inhibitory effects of ITCs on promotion and progression of carcinogenesis. Other chemopreventive mechanisms include induction of apoptosis, prevention of neoangiogenesis, anti-migratory or epigenetic effects. Scientific evidence of antitumor potential of ITCs provides a rationale for their use as chemopreventive agents. 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. 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.

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. 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 - glucosinaturtin, is major bioactive of watercress.

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 mM), streptomycin (100 mg L⁻¹), penicillin (100 IU mL⁻¹) and grown at 37°C in atmosphere with 5% CO₂, 95% air and 95% relative humidity.

Stock solutions of ITCs (Sigma–Aldrich, Germany) were made in dimethyl sulfoxide (DMSO) at concentrations of 28.3 mol.

mass fraction of 10%) and 50 µL of the obtained working so-
ent medium supplemented with fresh AB+ human serum (a
ated with ITCs. Briefly, stock solutions were diluted in nutri-
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.

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 interfa-
ces were washed three times with Haemaccel® (aqueous so-
lution 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 trea-
ted with ITCs. Briefly, stock solutions were diluted in nutri-
ent medium supplemented with fresh AB+ human serum (a
mass fraction of 10%) and 50 µL of the obtained working so-
lutions 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 le-
vel 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 pro-
liferation 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 frac-
tion 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 con-
trols and wells containing ITCs in investigated concentrations
but void of cells were used as corresponding blanks. The ran-
ges of ITCs concentrations tested (0–50 µM for experiments
on cancer cell lines and 0–80 µM on PBMC) were defined ba-
sed on the preliminary experiments with the highest concentra-
tion 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. Briefly, 20 µL of 3-(4,5-di-
emethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT),
Sigma-Aldrich, Germany solution (5 µg L\(^{-1}\) in phosphate buffer-
sed 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 absorban-
cence 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 in-
vestigated 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 concentra-
tion 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 do-
se-response curves for each ITC on each cell type, as a con-
centration of a drug that inhibited cell survival by 50%.

Results

The results obtained for antiproliferative action of inve-
istigated 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\(_{50}\) valu-
es (Table 1) BITC was the most potent inhibitor of cell proli-
feration in all cell lines treated. The potencies of SFN and
PEITC were similar. The order of sensitivity of various hu-
man 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 po-
tent 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

<table>
<thead>
<tr>
<th>Cell line/compound</th>
<th>SFN (mmol·m⁻³)</th>
<th>BITC (mmol·m⁻³)</th>
<th>PEITC (mmol·m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>13.59 ± 1.53</td>
<td>5.04 ± 1.73</td>
<td>12.00 ± 1.97</td>
</tr>
<tr>
<td>Fem-x</td>
<td>6.67 ± 0.73</td>
<td>2.76 ± 0.58</td>
<td>6.22 ± 0.32</td>
</tr>
<tr>
<td>LS 174</td>
<td>16.09 ± 2.44</td>
<td>14.30 ± 5.16</td>
<td>18.23 ± 3.04</td>
</tr>
<tr>
<td>PBMC</td>
<td>29.34 ± 15.3</td>
<td>45.74 ± 18.9</td>
<td>23.41 ± 7.13</td>
</tr>
<tr>
<td>PBMC+PHA</td>
<td>15.30 ± 5.39</td>
<td>21.92 ± 4.63</td>
<td>30.87 ± 6.33</td>
</tr>
</tbody>
</table>

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. IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 curr-
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 IC₅₀ values obtained with PBMC or mitogen-treated PBMC and IC₅₀ values for investigated ITCs on malignant cells. The values obtained for SI are presented in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Cell lines/compound</th>
<th>SFN</th>
<th>BITC</th>
<th>PEITC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ PBMC / IC₅₀ HeLa</td>
<td>2.16</td>
<td>9.07</td>
<td>1.95</td>
</tr>
<tr>
<td>IC₅₀ PBMC+PHA / IC₅₀ HeLa</td>
<td>1.12</td>
<td>4.35</td>
<td>2.57</td>
</tr>
<tr>
<td>IC₅₀ PBMC / IC₅₀ Fem-x</td>
<td>4.39</td>
<td>16.57</td>
<td>3.76</td>
</tr>
<tr>
<td>IC₅₀ PBMC+PHA / IC₅₀ Fem-x</td>
<td>2.28</td>
<td>7.94</td>
<td>4.96</td>
</tr>
<tr>
<td>IC₅₀ PBMC / IC₅₀ LS 174</td>
<td>2.16</td>
<td>3.19</td>
<td>1.28</td>
</tr>
<tr>
<td>IC₅₀ PBMC+PHA / IC₅₀ LS 174</td>
<td>1.12</td>
<td>1.53</td>
<td>1.69</td>
</tr>
</tbody>
</table>

*SI towards malignant cell line are calculated as the ratio of the half-maximal inhibitory concentration (IC₅₀) value for PBMC (or PBMC+PHA) and IC₅₀ 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 38. 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 He-La, 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 39 or PBMCs, as proposed in our work. Prioritisation of PBMCs as a model was rationalised by important role of PBMC subpopulations in the immuno-

The obtained results showing lower IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 characteristic 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 35. It was shown on Hepa 1c1c7 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. 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/N, 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. 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. 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. 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 sulphydryl 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. 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. 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. 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_{2}O_{2}. 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. 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 limboproliferative 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 anti-tumor 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. 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 glucotropoelin 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 containing 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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REFERENCES


25. Bonnemais C, Eggleston IM, Hayes JD. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. Cancer Res 2001; 61(16): 6120–30.


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