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# Induction of UDP-glucuronosyltransferase 1A by naturally occurring phytochemicals in human hepatoma cells

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ABSTRACT

UDP-glucuronosyltransferases (UGTs) are important detoxification and drug-metabolizing enzymes, which catalyse the glucuronidation of exogenous and endogenous chemicals. The anti-carcinogenic activity of dietary phytochemicals is partly attributed to the induction of phase II enzymes, including UGT1A. Our earlier study showed that protocatechuic acid increased UGT activity in rat liver. A similar effect was observed for indole-3-carbinol and phenethyl isothiocyanate in rat liver. In this study we assessed the effect of protocatechuic acid, tannic acid, indole-3-carbinol and phenethyl isothiocyanate on the expression and protein level of UGT1A in hepatocellular carcinoma HepG2 cells. Cells were incubated with 2μM and 10μM of protocatechuic acid, tannic acid, or indole-3-carbinol and 1μM and 5μM of phenethyl isothiocyanate for 72 hours. Transcript level was measured by RT-PCR and protein level by the immunoblot assay. Treatment with protocatechuic acid, tannic acid, indole-3-carbinol and phenethyl isothiocyanate induced the expression and protein level of UGT1A. Phenethyl isothiocyanate increased the mRNA of UGT1A to the most extent (0.28–5.7 fold change, $p < 0.05$). Increased expression of UGT1A was accompanied by the enhancement of its protein level, with the exception of protocatechuic acid at the dose of 2μM. Overall, isothiocyanates and indoles were more potent as UGT inducers than phenolic acids. Collectively, the results suggest that the induction of UGT1A could contribute to the hepatoprotective and chemopreventive properties of these phytochemicals.

**Keywords:** tannic acid, protocatechuic acid, indole-3-carbinol, phenethyl isothiocyanate, UGT1A, HepG2.

## Introduction

Uridine diphosphoglucuronosyltransferases (UGTs) are phase II enzymes that conjugate metabolic intermediates with glucuronic acid to form glucuronides, which are characterized by increased solubility in water and therefore are more easily excreted from the body [1]. Two general classes of UGTs exist in humans: UGT1 and UGT2. Both classes contain multiple members that catalyze the glucuronidation of a diverse array of substrates and are differentially expressed in various organs [2]. UGT1A enzymes metabolize both endogenous and exogenous compounds such as bilirubin and drugs, whereas UGT2B enzymes target rather endogenous compounds such as steroid hormones [3].

Several studies demonstrated that diets containing plant foods, particularly cruciferous vegetables induce drug metabolizing enzymes in humans and rodents [4–7]. Glucosinolates present in *Cruciferae* vegetables are hydrolyzed by myrosinase during food processing to biologically active indoles and isothiocyanates. The structure of these phytochemicals is shown in **Figure 1**. In 1997, Taioli et al. [8] reported the induction of UGT activity in humans fed 400mg of indole-3-carbinol for 5 days. Other studies showed that indole-3-carbinol increased UGT activity in male Wistar rats [9]. Phenethyl isothiocyanate increased hepatic UGT activity in male Fisher 344 rats [10]. A similar effect has been observed in the case of phenolic acids, which are prev-

alent among many edible plants. Protocatechuic acid, a simple phenolic acid (**Figure 1**) increased UGT activity in the epidermis of female Swiss mice [11]. On the other hand, tannic acid, which is a mixture of digallic acid esters of glucose, did not significantly change hepatic activity of UGT in Wistar rats [12]. These studies indicate that phytochemicals may increase the activity of UGT in *in vivo* models. Thus, the up-regulation of this enzyme by phenolic acids, indoles and isothiocyanates may also represent one of the cancer preventive mechanisms of these compounds.

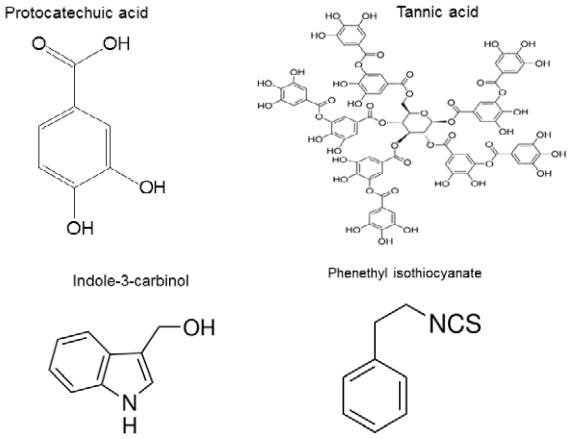


**Figure 1.** Chemical structure of protocatechuic acid, tannic acid, indole-3-carbinol and phenethyl isothiocyanate

Liver is the major organ for glucuronidation of a variety of xenobiotics. Carcinogens, including benzo(a)pyrene, are conjugated by UGT1A, and increased glucuronidation would result in their increased elimination and potentially decreased toxicity [13]. In this regard, the effective neutralization of chemical mutagens may significantly reduce the risk of cancer initiating events.

Therefore, the objective of this study was to evaluate the effects of phytochemicals such as tannic acid, protocatechuic acid, indole-3-carbinol and phenethyl isothiocyanate on the expression of UGT in human hepatocellular carcinoma HepG2 cells.

## Material and methods

### Chemicals and antibodies

Antibiotics solution ($10^4$U penicillin, 10 mg streptomycin, 25µg amphotericin B), dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), Radio-Immunoprecipitation Assay (RIPA) buffer, Tris were obtained from Sigma-Aldrich (St. Louis, MO, USA). HepG2 (ATCC HB 8065) cells were provided by Prof. Zofia Mazerska from the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Poland. All the primers used in PCR reactions were obtained from oligo.pl (Warsaw, Poland). Protease inhibitor cocktail was obtained from Roche Diagnostics GmbH (Penzberg, Germany). Primary and secondary antibodies against UGT and β-actin were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rainbow colored protein molecular weight marker was purchased from Amersham Pharmacia Biotechnology (Piscataway, NJ, USA). All the other chemicals were commercial products of the highest purity available.

### Cell culture and treatment

HepG2 cells were maintained in DMEM containing 10% fetal bovine serum and antibiotics. The cells were grown in a humidified incubator at 37°C in the atmosphere of 5% $CO_2$. To assess the effect of tannic acid, protocatechuic acid, indole-3-carbinol and phenethyl isothiocyanate on the measured parameters, $5x10^5$ cells were seeded per 100mm culture dish. After 24 hours of initial incubation the cells were treated with 2µM and 10µM tannic acid, protocatechuic acid or indole-3-carbinol and 1µM and 5µM phenethyl isothiocyanate or 0.1% vehicle control. The incubation was continued for subsequent 72 hours and cells were harvested.

### RNA isolation and total protein lysates preparation

Extraction of total RNA from cells was performed using GeneMatrix Universal DNA/RNA/Protein Purification Kit (EurX, Poland) according to the manufacturer's instructions. Whole cell lysates were prepared using RIPA buffer.

### Quantitative PCR

Total RNA was subjected to reverse transcription using RevertAid Kit (Fermentas, Burlington, Canada) followed by quantitative real-time PCR. For real-time analyses the Maxima SYBR Green Kit (Fermentas) and a BioRad Chromo4 thermal cycler were used. The protocol started with 5 minutes enzyme activation at 95°C, followed by 40 cycles of 95°C for 15 s, 54°C for 20 s and 72°C for 40 s and final elongation at 72°C for 5 minutes. Melting curve analysis was used for product verification. The estimation of the expression of *TBP* (TATA box binding protein) and *PBGD* (porphobilinogen deaminase) was used for data normalization. Primer sequences for UGT1A transcript analysis were as follows: 5'-AACGATCTGCTTGGTCAC-3' (forward) and 5'-GAACATTCAGGGTCACTCC-3' (reverse).

### Western blot analysis

For the determination of the level of UGT protein the immunoblot assay was used. Protein content in the samples was determined by the Lowry method [14]. All the experiments were repeated three times. Whole cell lysate samples containing 100μg proteins were separated in 10% SDS-PAGE slab gels and proteins were transferred to nitrocellulose membranes. After blocking with 10% skimmed milk, proteins were probed with goat polyclonal UGT and/or rabbit polyclonal β-actin antibodies. Estimation of the level of β-actin was used as an internal control. As the secondary antibodies in the staining reaction, the peroxidase-labeled anti-goat IgG or anti-rabbit IgG were used. Bands were visualized with Advansta Western Bright Quantum Western blotting detection kit. The amount of the immunoreactive product in each lane was determined using the Quantity One software (BioRad Laboratories, Hercules, CA, USA). Values were calculated as relative absorbance units (RQ) per mg protein.

## Statistical analysis

The statistical analysis was performed by one-way ANOVA. The statistical significance between the experimental groups and their respective controls was assessed by Tukey's post hoc test, with $p < 0.05$.

## Results

### Analysis of the expression of UGT1A

The expression of *UGT1A* was measured by quantitative PCR (**Figure 2**). The expression of *UGT1A* mRNA increased after treatment of HepG2 cells with all the tested compounds. *UGT1A* mRNA level was enhanced by (0.28–5.7-fold) after treatment with either dose of phenethyl isothiocyanate. Indole-3-carbinol in either dose induced about 1.14–1.83-fold higher expression of UGT1A. Tannic acid in either dose increased about 0.48–0.96-fold the expression of UGT1A, while protocatechuic acid increased about 0.27-fold the expression of UGT only in the higher dose.

### Evaluation of the protein level of UGT1A

The level of UGT1A protein in HepG2 cells was investigated using the Western blot assay with a specific antibody against the enzyme (**Figure 3**). The content of UGT1A (**Figure 3**) increased by about 28–75% after the treatment with either phenethyl isothiocyanate or indole-3-carbinol in HepG2 cells. The level of UGT1A increased by 26–45% after the treatment with 2 and 10μM tannic acid and 10μM protocatechuic acid in HepG2 cells. No statistically significant changes in the content of the analyzed protein were detected in HepG2 cells as the result of treatment with the protocatechuic acid at the dose of 2μM (**Figure 3**).

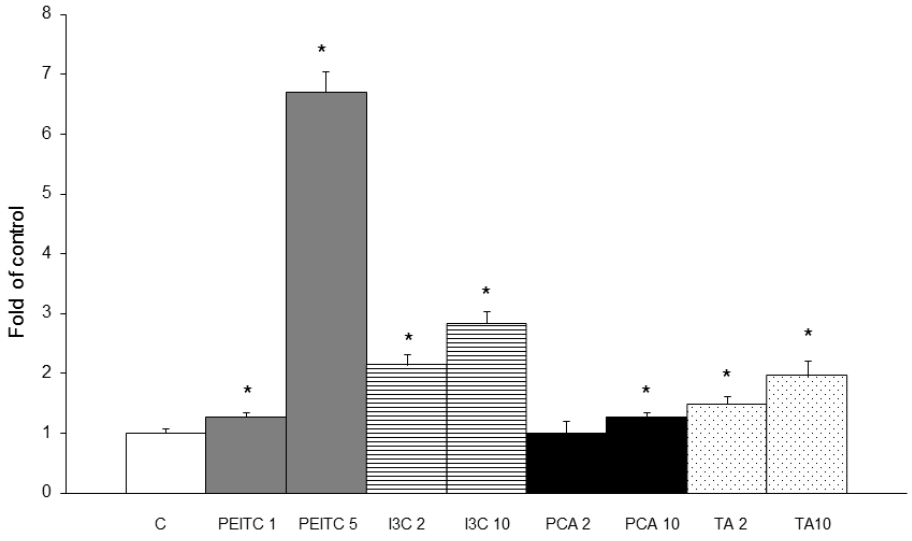


**Figure 2.** The effect of phenethyl isothiocyanate, indole-3-carbinol, protocatechuic acid and tannic acid on UGT1A transcript level in HepG2 cells. Data (mean±SEM) from three separate experiments are presented, the asterisk above the bar denotes statistically significant differences from the control group, $p < 0.05$
*C: vehicle control; PEITC: phenethyl isothiocyanate; I3C: indole-3-carbinol; PCA: protocatechuic acid; TA: tannic acid*

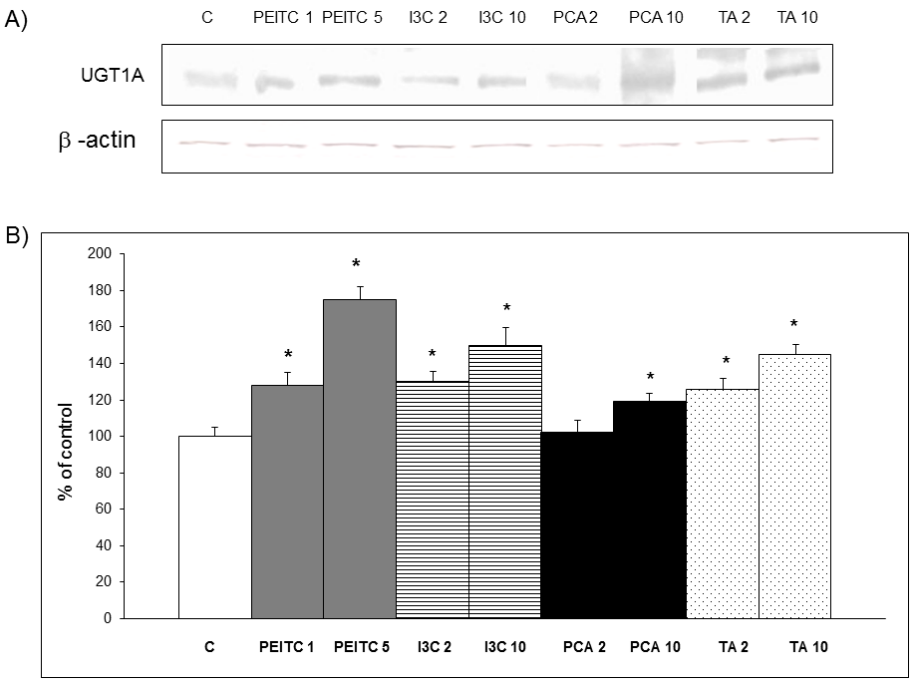


**Figure 3.** The effect of phenethyl isothiocyanate, indole-3-carbinol, protocatechuic acid and tannic acid on the level of the UGT1A protein in HepG2 cells. (A) Representative immunoblots for the analysis of UGT1A protein; β-actin was used as a normalization control. (B) Results of Western blot analysis of the content of UGT1A protein in comparison to the control group. Data (mean±SEM) from three separate experiments are presented, the asterisk above the bar denotes statistically significant differences from the control group, $p < 0.05$

## Discussion

It is well known that dietary factors affect the development of human cancers. The human diet contains a large number of a variety of compounds that may inhibit mutagenesis and/or carcinogenesis [15–18]. These compounds are very diverse in chemical structures and their protective mechanisms are generally unclear. Although the prevention of cancer may be due to multiple mechanisms, one mode of action of anti-carcinogens may be to enhance the carcinogen detoxification systems, such as UGT [19]. UGT can minimize carcinogenicity by conjugation of chemicals with glucuronic acid, which in general results in the generation of biologically less active molecules and increases water-solubility of the conjugated products, which facilitates their excretion from the body via bile or urine [20]. Therefore, the enhancement of the activity of UGT could potentially augment the capacity to withstand the burden of toxic agents and (pre)carcinogens to wchich humans are exposed daily [21]. In this regard, it was shown that UGT1A-deficient rats show reduced capacity to glucuronidate benzo(a)pyrene, a mutagenic polycyclic aromatic hydrocarbon and are more susceptible to formation of DNA adducts [22, 23]. In addition, Berges et al. [24] reported that UGT activity was inversely correlated with the number of preneoplastic liver foci in rats, supporting a role of UGT induction in cancer chemoprevention.

Increased glucuronidation could mediate the protective and antioxidant effects of chemopreventive compounds. Several studies which were performed in animals models indicated that naturally occurring dietary anticarcinogens may be able to elevate UGT activity [25, 26]. In particular, isothiocyanates such as sulforaphane and phenethyl isothiocyanate, that exhibit chemopreventive properties have been shown to induce UGT in animal models [27, 28]. In addition, whole tea, tea extracts, and polyphenols from different types of tea also induce UGT activity and/or expression in animals and cell culture systems [29]. Moreover, our previous study showed that simple phenolic acid, protocatechuic acid increased the activity of UGT in rat liver [30].

In this study, we evaluated the ability of different plant phenolic acids and glucosinolates to modulate the UGT1A expression and protein level in HepG2 hepatoma cell line. Both phenolic acids induced *UGT1A* transcript in HepG2 cells. Tannic acid had a stronger effect on UGT1A than a simple phenolic acid, protocatechuic acid. However, the most potent inducers of UGT1A

were glucosinolates: indole-3-carbinol and phenethyl isothiocyanate. Western blot analysis confirmed that the accumulation of transcript was accompanied by an increase in UGT1A protein level.

Our study showed that phenethyl isothiocyanate induced the expression and protein level of UGT1A to the most extent. A similar effect was observed by Sertzer et al. [9] and van der Loght [12] in rat liver. In contrast to our results, van der Loght et al. [12] observed no effects on hepatic UGT activity after treatment with indole-3-carbinol. This discrepancy may be related to differences in doses and/or species-specific effects.

The inducers of xenobiotic metabolism enzymes can be divided into monofunctional and bifunctional modulators [31]. Isothiocyanates are considered as monofunctional modulators since they induce the activity of only phase II enzymes. In our previous studies we confirmed that phenethyl isothiocyanate was the most effective inducer of phase II enzymes such as NQO1 and GST in rat liver [32]. This study shows that isothiocyanates and indoles are more active as inducers of phase II enzymes than phenolic acids in human hepatoma cells. Collectively, the results suggest that the induction of UGT1A may be one of the factors contributing to the hepatoprotective and chemopreventive properties of phenethyl isothiocyanate, indole-3-carbinol and, to a lesser extent, tannic acid and protocatechuic acid. Further studies are necessary to establish the direct relevance of the studied compounds in providing health benefits for humans.


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### Conflicts of interest statement

*All the authors declare no conflicts of interest.*

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