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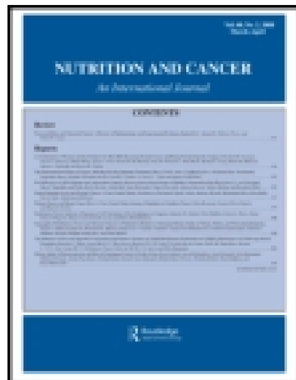

Nutrition and Cancer
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hnuc20

Modulation of CYP1A1, CYP1A2 and CYP1B1 Expression by Cabbage Juices and Indoles in Human Breast Cell Lines

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Version of record first published: 20 Jun 2012.

To cite this article: Hanna Szaefer, Barbara Licznerska, Violetta Krajka-Kuźniak, Agnieszka Bartoszek & Wanda Baer-Dubowska (2012): Modulation of CYP1A1, CYP1A2 and CYP1B1 Expression by Cabbage Juices and Indoles in Human Breast Cell Lines, Nutrition and Cancer, 64:6, 879-888

To link to this article: http://dx.doi.org/10.1080/01635581.2012.690928

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Nutrition and Cancer, 64(6), 879–888
Copyright © 2012, Taylor & Francis Group, LLC
ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635581.2012.690928

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Modulation of CYP1A1, CYP1A2 and CYP1B1 Expression by Cabbage Juices and Indoles in Human Breast Cell Lines

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Epidemiological studies have shown that consumption of cabbage and sauerkraut is connected with significant reduction of breast cancer incidences. Estrogens are considered a major breast cancer risk factor and their metabolism by P450 enzymes substantially contributes to carcinogenic activity. The aim of this study was to investigate the effect of cabbage and sauerkraut juices of different origin on the expression profile of the estrogen metabolism key enzymes (CYP1A1, CYP1A2, CYP1B1) in breast cell lines MCF7, MDA-MB-231, and MCF10A. The effects of cabbage juices were compared with that exerted by indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM). The treatment with cabbage juices or indoles for 72 h affected the expression of CYP1 family genes in cell-type dependent manner. Their induction was found in all cell lines, but the ratio of CYP1A1 to CYP1B1 was 1.22- to 10.6-fold in favor to CYP1A1 in MCF7 and MCF10A cells. Increased levels of CYP1A2 in comparison with CYP1B1 were also observed in MCF7 cells. In contrast, in MDA-MB-231 cells CYP1B1 was preferentially induced. Since the cell lines investigated differ in invasion capacity, these results support epidemiological observations and partly explain the mechanism of the chemopreventive activity of white cabbage products.

INTRODUCTION

Estrogens are considered the prime risk factor of breast cancer (1). Prolonged exposure to estrogens may not only promote cancer development, but also initiate carcinogenesis through the reaction of their active metabolites with DNA. One possible mechanism of DNA damage caused by estrogens may be explained by the unique chemical structure of estrogens. Unlike all other steroid hormones, estrogens such as 17β-estradiol have an aromatic A-ring, which yields catechols upon oxidation that may be further oxidized to highly reactive semiquinones and quinones (2). The labile quinines (e.g., 4-hydoxyestradiol-quinone) form adducts such as the apurinic 4-hydroxyestradiol-1(α,β)-N^7-guanine and 4-hydroxyestradiol-1(α,β)-N^3-adenine (2–4). Thus estrogen quinones seem to share a common feature of many chemical carcinogens (i.e., the ability to covalently modify DNA). The further support of this concept comes from the experiments on animal models as well as the analysis of human breast tissues (5,6).

The oxidative metabolism of estrogens is mainly catalyzed by cytochromes P450, CYP1A1, CYP1A2, and CYP1B1. The latter is highly expressed in estrogen target tissues, including mammary gland, and specifically catalyzes the 4-hydroxylation of estradiol (7). Then 4-hydroxyestradiol can either be converted into 4-methoxyestradiol by catechol-*O*-methyltransferase or undergo redox cycling resulting in the above mentioned quinones and semiquinones. CYP1B1 is also expressed in diverse types of human cancer including breast tumor (8). These reports strongly suggest that the specific and local formation of 4-hydroxylation of estradiol is important for breast carcinogenesis, and imply CYP1B1 as a key player in this process. In contrast to 4-hydroxyestradiol, 2-hydroxyestradiol, which is formed in the reaction catalyzed by CYP1A2 in the liver and by CYP1A1 in the extrahepatic tissues, is not considered carcinogenic. It is known that 2-hydroxyestradiol is methylated by catechol-*O*-methyltransferase at a higher rate than 4-hydroxyestradiol. Thus carcinogenic free radicals are not easily generated (7).

The potential roles of CYP1A1, CYP1A2 and CYP1B1 in carcinogenesis and tumor progression have led to the development of specific modulators of these enzymes as potential anticarcinogenic agents including that targeting breast cancer (9).

Submitted 21 November 2011; accepted in final form 16 April 2012.
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Since the expression of CYP1A1 and CYP1B1 depends on cell-specific factors in human breast cancer cell lines, particularly estrogen receptor (ER) status (10), the inhibitors targeting both CYPs and ER are particularly desirable.

Although there are pharmaceutical agents known to play therapeutic and preventive roles in breast cancer, the use of compounds from natural products to prevent breast cancer is currently being explored. Among the promising food components investigated to reduce breast cancer risk, there are phytochemicals found in cruciferous vegetables: indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM). I3C is an autolysis product of glucosinolate, glucobrassicin, which is found in Brassica vegetables such as cabbage, broccoli, and Brussels sprouts. It has been shown that I3C and its an acid-catalyzed condensation product DIM exhibit anticarcinogenic properties in laboratory animals and inhibit the growth of human cancer cells (11,12). It is supposed that antitumor properties of I3C in breast cancer are related, in part, to its ability to alter estrogen metabolism in vitro and in vivo (12).

In the Central and Eastern European diet, the most common *Brassica* genus is white cabbage and its fermented product, sauerkraut. However, in contrast to the other *Cruciferae* representatives, anticarcinogenic activity of white cabbage and sauerkraut have been less extensively studied. Epidemiological studies have shown, however, that high intake of white cabbage may be associated with a lower risk of breast cancer (13).

The aim of the present study was to evaluate the effect of cabbage juices on the expression profile of CYP1A1, CYP1A2, and CYP1B1 in breast epithelial cell lines differing in ER status, tumorigenic (MCF7, MDA-MB-231) and nontumorigenic (MCF10A). The effects were compared with those of the commercially available glucosinolates (GLS) breakdown products: I3C and DIM.

MATERIALS AND METHODS

Chemicals

Indole-3-carbinol, dithiothreitol, antibiotics solution (10^4U penicillin, 10 mg streptomycin, 25 μg amphotericin B), bovine serum albumin, dimethyl sulfoxide (DMSO), fetal bovine serum, Dulbecco's Modified Eagle's Medium (DMEM), hydrocortisone, 10 mg/ml insulin, 100 μg/ml epidermal growth factor (EGF), horse serum, RIPA buffer, Tris, tRNA from *E. coli*, were purchased from Sigma Chemicals Co. (St. Louis, MO). 3,3′-diindolylmethane was obtained from LKT Laboratories (St. Paul, MN). Primary antibodies against CYP1A1, CYP1A2, and β-actin and secondary antibodies were supplied by Santa Cruz Biotechnology (Santa Cruz, CA). Primary and secondary antibodies against CYP1B1 purified standard CYP1B1 were obtained from BD Biosciences (Woburn, MA). All the antibodies used in these experiments were specific for their respective proteins, and according to the information provided by suppliers, there was no cross-reactivity within the isozymes of the same family. Protease inhibitor tablets were obtained from Roche Diagnostics GmbH (Penzberg, Germany). All other chemicals were commercial products of the highest purity available. I3C and DIM were dissolved in DMSO at a concentration of 100 mM and stored at −20°C.

Preparation of Juices

Fresh white cabbage was purchased in a wholesale shop supplying the area of Gdansk (Poland) in vegetables and from the organic farm FOHAT (certificate N°92042A). The juice preparation and standardization was performed as previously described (14,15). In fresh juice, the average content of GLS was 3.283–4.623 μmol/g of dry mass and of I3C was 11.37–14.81 μmol/L. In sauerkraut juice, the content of most GLS was below the level of detection and I3C was 20.53–32.10 μmol/L. Before addition to the cell culture medium, sauerkraut juices were neutralized with NaOH to pH ∼7.4 and sterilized by filtration through Ø22 μm filters.

Cell Culture and Treatment

MCF7 (ECACC 86012803) and MDA-MB-231 (ECACC 92020424) cells were purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). The MCF10A (ATTC CRL-10317) cell line was a gift from Dr Blazej Rubis (Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, Poznań, Poland). MCF7 and MDA-MB-231 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics solution. To assess the effects of the compounds tested, the cells were grown in the presence of 5% fetal bovine serum. MCF10A cells were cultured in DMEM supplemented with 0.1% insulin solution, 0.02% EGF solution, 0.05% hydrocortisone solution, 5% horse serum, and 1% antibiotics solution. All cell lines were routinely maintained in T75 flasks at 37°C in a humidified environment of 5% CO_2/95% air and were passed twice a week using 0.05% trypsin/0.02% EDTA. Experiments were conducted at a cell density of 70% confluency. After 24-h preincubation, the cells were treated with raw cabbage or sauerkraut juices, I3C and DIM at the doses selected on the basis of the viability assay as previously described (16). The incubation was continued for subsequent 72 h. Control cells were treated with vehicle (DMSO or water). The concentration of DMSO did not exceed 0.1%.

Real-Time PCR

Total RNA was isolated using the GenElute Mammalian Total RNA Miniprep Kit (Sigma, St. Louis, MO) according to manufacturer's recommendations and subjected to reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Fermantas, St. Leon-Rot, Germany), followed by quantitative real-time PCR. For real-time analyses, the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas) and BioRad Chromo4 were used. The protocol started with a 5-min enzyme activation at 95°C, followed by 40 cycles of 95°C for 15 s, 56°C

for 20 s, 72°C for 40 s, and the final elongation at 72°C for 5 min. The melting curve analysis was used for product size verification. Experiments were normalized for the expression of TATA box binding protein (TBP) and porphobilinogen deaminase (PBGD). The Pfaffl relative method was used for fold-change quantification. The following primers were used for forward and reverse: CYP1A1 (5′GAACCTTCCCTGATCCTTGTG3′ and 5′GAGGTGGCTGAGGTACTGAG3′); CYP1A2 (5′AGAG GTTCCTGTGGTTCC3′ and 5′CCCTTCTTGCTGTGCTT G3′); CYP1B1 (5′GCCACTATCACTGACATC3′ and 5′GAC CTGATCCAATTCTGC3′); PBGD (5′TCAGATAGCATACAA GAGACC3′ and 5′TGGAATGTTACGAGCAGTG3′); and TBP (5′GGCACCACTCCACTGTATC3′ and 5′GGGATTATATTCG GCGTTTCG3′).

Protein Immunoblotting

The adherent and floating cells were harvested, lysed in RIPA buffer supplemented with proteinase inhibitors, and incubated on ice for 60 min. Cell lysate was centrifuged at 14,000 rpm for 15 min and the supernatant was recovered. Equal amounts of protein (100 μg) were subjected to 10% SDS-PAGE slab gels and transferred to nitrocellulose membranes (Immobilon-P; Millipore, Bedford, MA) according to the method of Laemmli (17) and Towbin (18). After blocking with 5% or 10% skimmed milk, the proteins were probed with goat polyclonal CYP1A1, goat polyclonal CYP1A2, rabbit antirat CYP1B1, and rabbit antimouse β-actin antibodies. As the secondary antibodies, the alkaline phosphatase-labeled antigoat IgG or antirabbit IgG were used. Protein contents were measured using albumin as a standard and the β-actin protein as an internal control. The amount of immunoreactive product in each lane was determined by densitometric scanning using a BioRad GS710 Image Densitometer (BioRad Laboratories, Hercules, CA). The values were calculated as relative absorbance units (RQ) per mg protein.

Statistical Analysis

Statistical analysis was performed by 1-way ANOVA. The statistical significance between the experimental groups and their respective controls was assessed by Tukey's post hoc test, at $P < 0.05$.

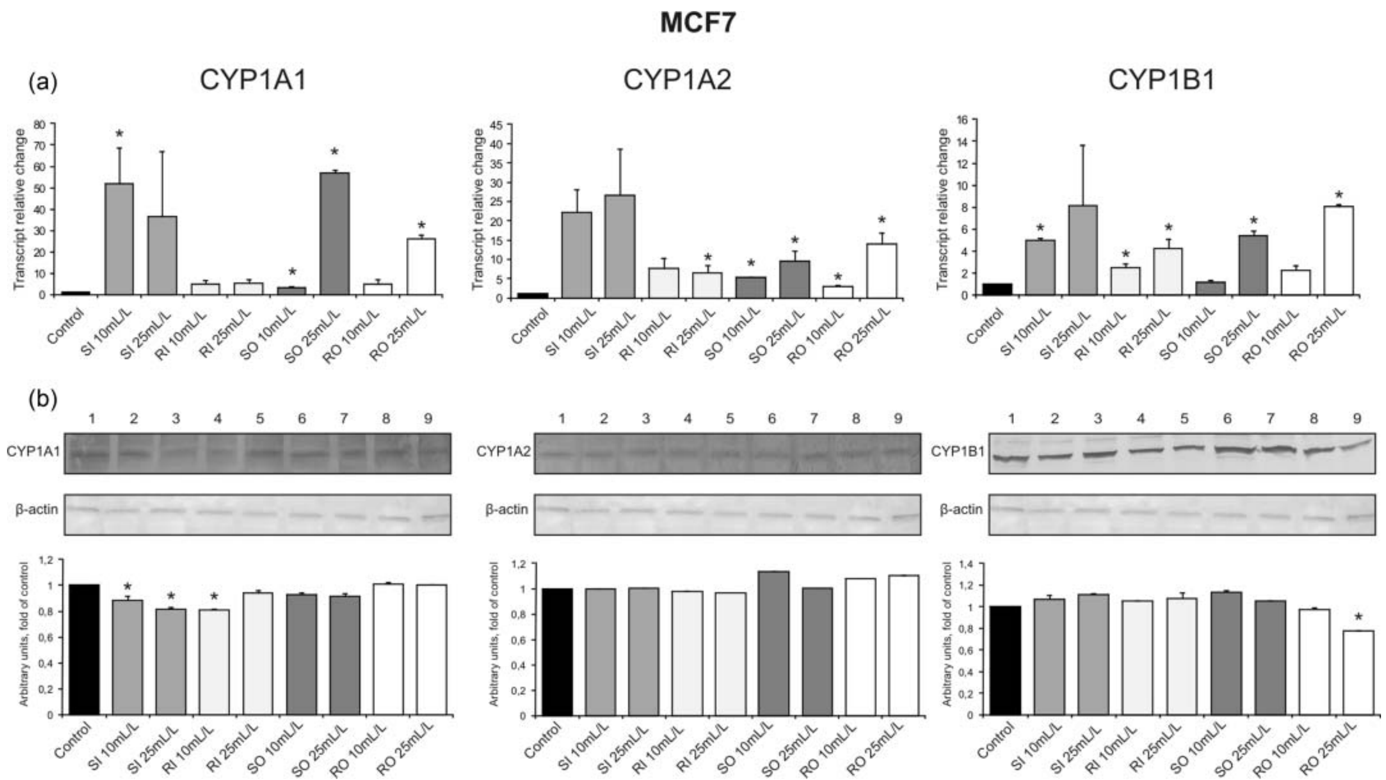

FIG. 1. The effect of 72-h incubation with cabbage juices on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MCF7 cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); sauerkraut juice from industrial farming (SI) 10 mL/L (lane 2); SI 25 mL/L (lane 3); raw juice from industrial farming (RI) 10 mL/L (lane 4); RI 25 mL/L (lane 5); sauerkraut juice from organic farming (SO) 10 mL/L (lane 6); SO 25 mL/L (lane 7); raw juice from organic farming (RO) 10 mL/L (lane 8); RO 25 mL/L (lane 9). SI = sauerkraut juice from industrial farming; RI = raw juice from industrial farming; SO = sauerkraut juice from organic farming; RO = raw juice from organic farming.

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RESULTS

The Effect of Cabbage Juices and Indoles on the Expression of CYP1A1, CYP1A2, and CYP1B1 in MCF7 Cells

As expected, constitutive expression of all CYP1 genes tested was very low in all breast cell lines investigated. However, the constitutive expression of CYP1B1 was slightly higher than that of CYP1A1 and CYP1A2 (data not shown). Treatment with cabbage juices, particularly with a sauerkraut juice for 72 h, increased the most the CYP1A1 transcript in this cell line (by up to 57-fold), followed by CYP1A2 (up to 14-fold) and CYP1B1 (up to 8-fold). The CYP1 protein level, however, in most cases was unaffected with the exception of sauerkraut juice from industrial farming in both tested doses and raw juice from industrial farming in the lower dose, which decreased the CYP1A1 protein levels. The CYP1B1 protein level was also decreased as result of treatment with the juice obtained from the cabbage grown in the organic farm applied at a higher dose (Fig. 1).

Similarly to cabbage juices, I3C caused ∼62-fold increase in the CYP1A1 transcript and this effect was dose-dependent. The transcripts of CYP1A2 and CYP1B1 were also increased but only ∼11–12-fold and for CYP1A2 the observed changes were not statistically significant. The effect of DIM on CYP1A1 and CYP1A2 transcript levels was less pronounced than that of I3C, but comparable, particularly at a lower dose on CYP1B1. The doses of both indoles, although different, corresponded to their nontoxic concentrations (viability above 70% based on MTT assay) (16). CYP1A2 and CYP1B1 protein levels were not changed, whereas CYP1A1 was unaffected (30 μM of I3C) or diminished (50 μM of I3C and both doses of DIM) (Fig. 2).

The Effect of Cabbage Juices and Indoles on the Expression of CYP1A1, CYP1A2, and CYP1B1 in MDA-MB-231 Cells

Fig. 3 shows the data on the effect of cabbage juices and indoles on CYP1 genes expression in estrogen-negative invasive MDA-MB-231 cells. Treatment of these cells with cabbage juices had no effect, slightly decreased (raw juice from industrial farming at the dose of 2.5 mL/L) or increased CYP1A1 transcripts (raw and sauerkraut juice from organic farming, respectively), leaving protein level unchanged. The CYP1A2 protein level was slightly increased as a result of treatment with the

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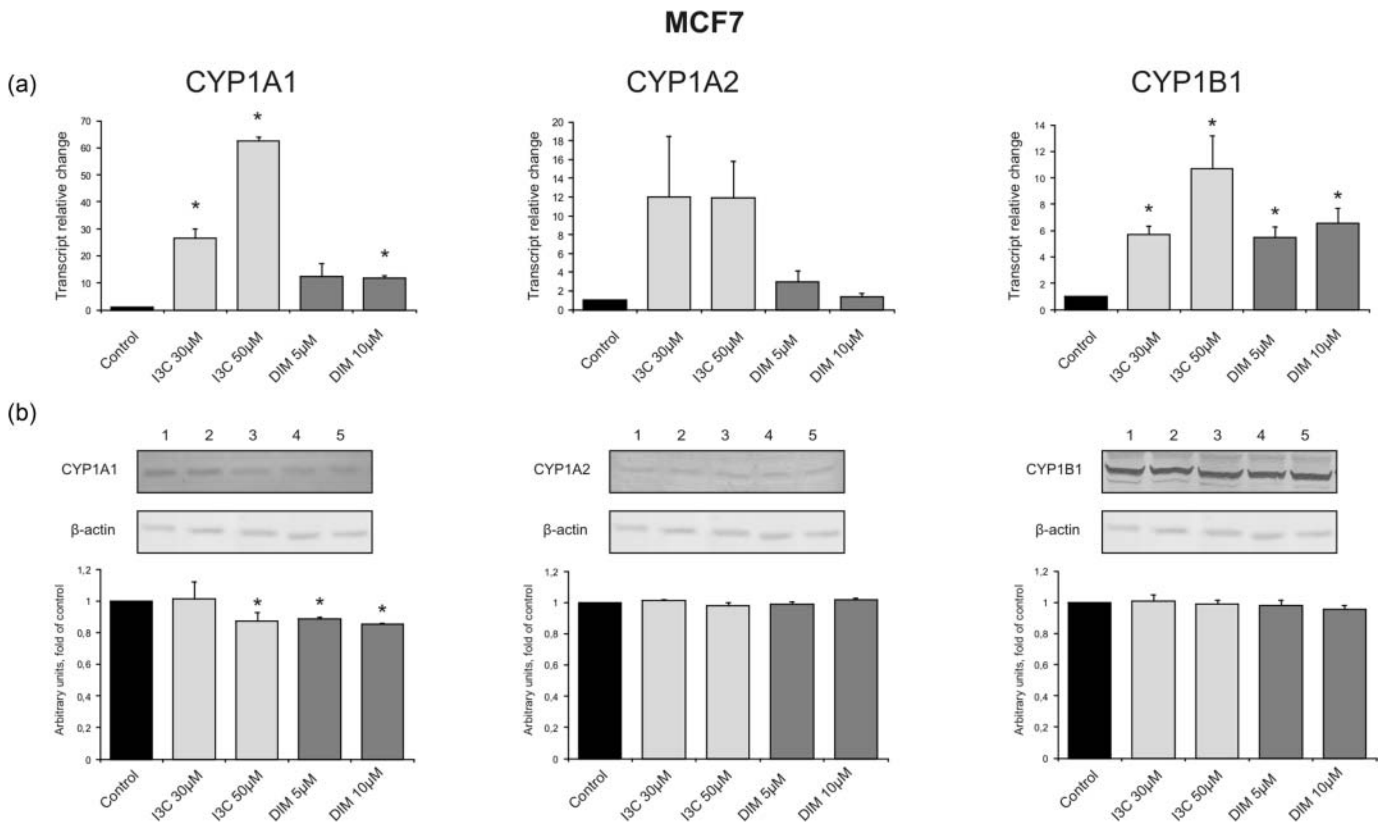

FIG. 2. The effect of 72-h incubation with indoles on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MCF7 cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); Indole-3-carbinol (I3C) 30 μM (lane 2); I3C 50 μM (lane 3); 3,3′-diindolylmethane (DIM) 5 μM (lane 4); DIM 10 μM (lane 5). I3C = indole-3-carbinol; DIM = 3,3′-diindolylmethane.

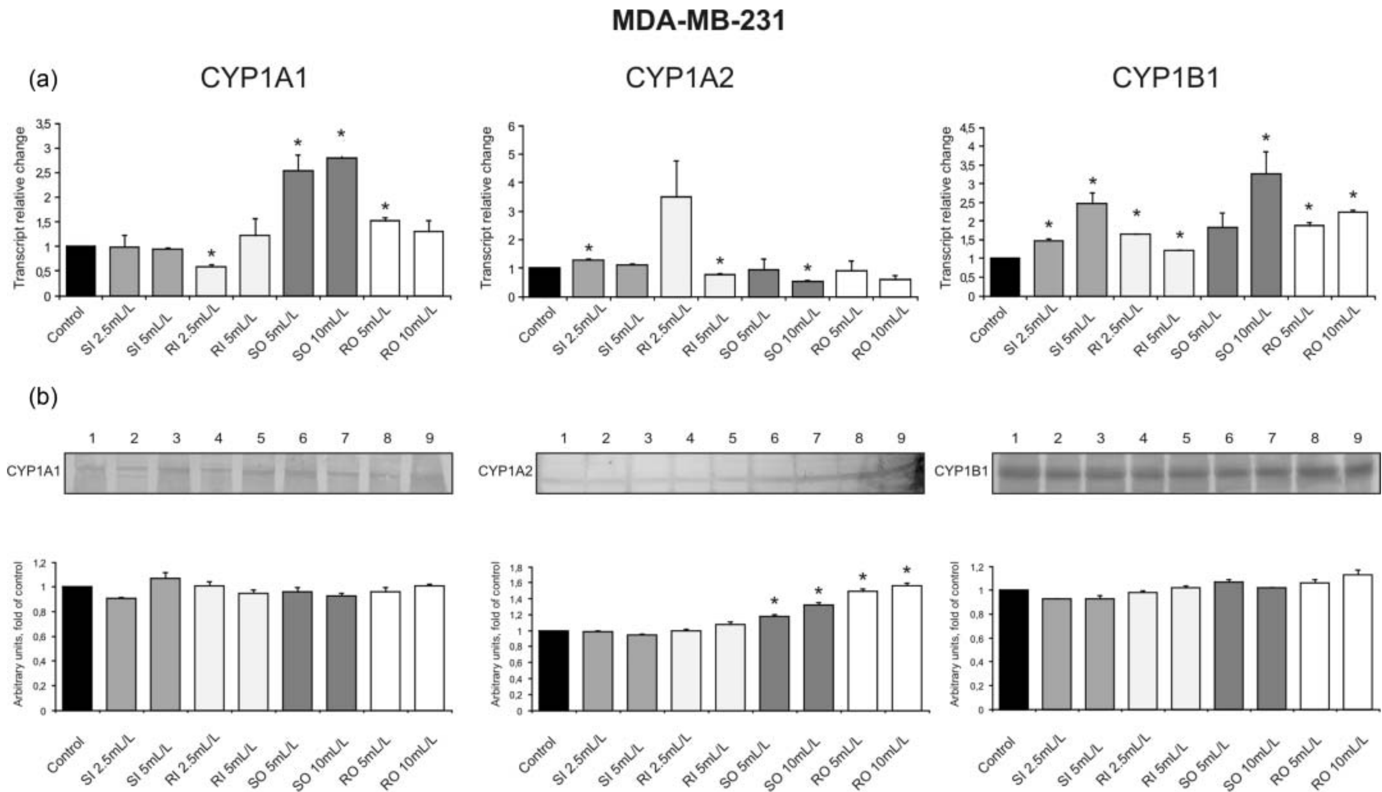

FIG. 3. The effect of 72-h incubation with cabbage juices on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MDA-MB-231 cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); SI 2.5 mL/L (lane 2); SI 5 mL/L (lane 3); RI 2.5 mL/L (lane 4); RI 5 mL/L (lane 5); SO 5 mL/L (lane 6); SO 10 mL/L (lane 7); RO 5 mL/L (lane 8); RO 10 mL/L (lane 9). SI = sauerkraut juice from industrial farming; RI = raw juice from industrial farming; SO = sauerkraut juice from organic farming; RO = raw juice from organic farming.

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same juices obtained from cabbage grown in the organic farm, although its mRNA level was decreased or unaffected. The most significant increase in this cell line as a result of the treatment with cabbage juices was observed in the expression of CYP1B1 but only at mRNA level.

As shown in Fig. 4, IC3 increased the most in the CYP1A1 transcript (∼16-fold at the dose of 30 μM), but CYP1A2 and CYP1B1 mRNA were also increased as a result of treatment with this indole but only 5- and 6-fold, respectively. In contrast to I3C, DIM increased the most in the CYP1B1 transcript in this cell line (5-fold at the dose of 5 μM).

The Effect of Cabbage Juices and Indoles on the Expression of CYP1A1, CYP1A2, and CYP1B1 in MCF10A Cells

MCF10A cell line represents immortalized nontumorigenic epithelial breast cells. Treatment of these cells with cabbage juices, particularly sauerkraut, both derived from cabbage grown on industrial and organic farm enhanced the expression of CYP1A1, although only on mRNA level (up to ∼7-fold). Treatment of these cells with sauerkraut juice derived from cabbage grown on organic farm also caused an increase in the CYP1A2 transcript. The expression of CYP1B1 was increased the most by the juices obtained from raw cabbage grown on the industrial or organic farms, used in higher doses (Fig. 5).

Similar to sauerkraut juices, indoles, I3C, and DIM enhanced the most the expression of CYP1A1. However, I3C increased both CYP1A1 mRNA and protein level, but only at the higher dose, whereas treatment with DIM slightly decreased this CYP protein level. The slight increase in CYP1B1 transcripts was also observed as a result of the treatment by both indoles (Fig. 6).

The Evaluation of the Relationship Between CYP1A1, CYP1A2, and CYP1B1 mRNA Expression in Response to Cabbages Juices and Indoles Exposure

To compare the relationship between CYP1A1, CYP1A2, and CYP1B1 in response to the treatment with cabbage juices and indoles in the cell lines investigated, the ratios of CYP1A1 and CYP1A2 to CYP1B1 fold changes were calculated for each dose and type of treatment. The ratios greater than 1 indicate that the expression of CYP1A1 or CYP1A2 was induced to higher level than the expression of CYP1B1. As shown in Table 1, the treatment of MCF7 and MCF10A with raw cabbage or

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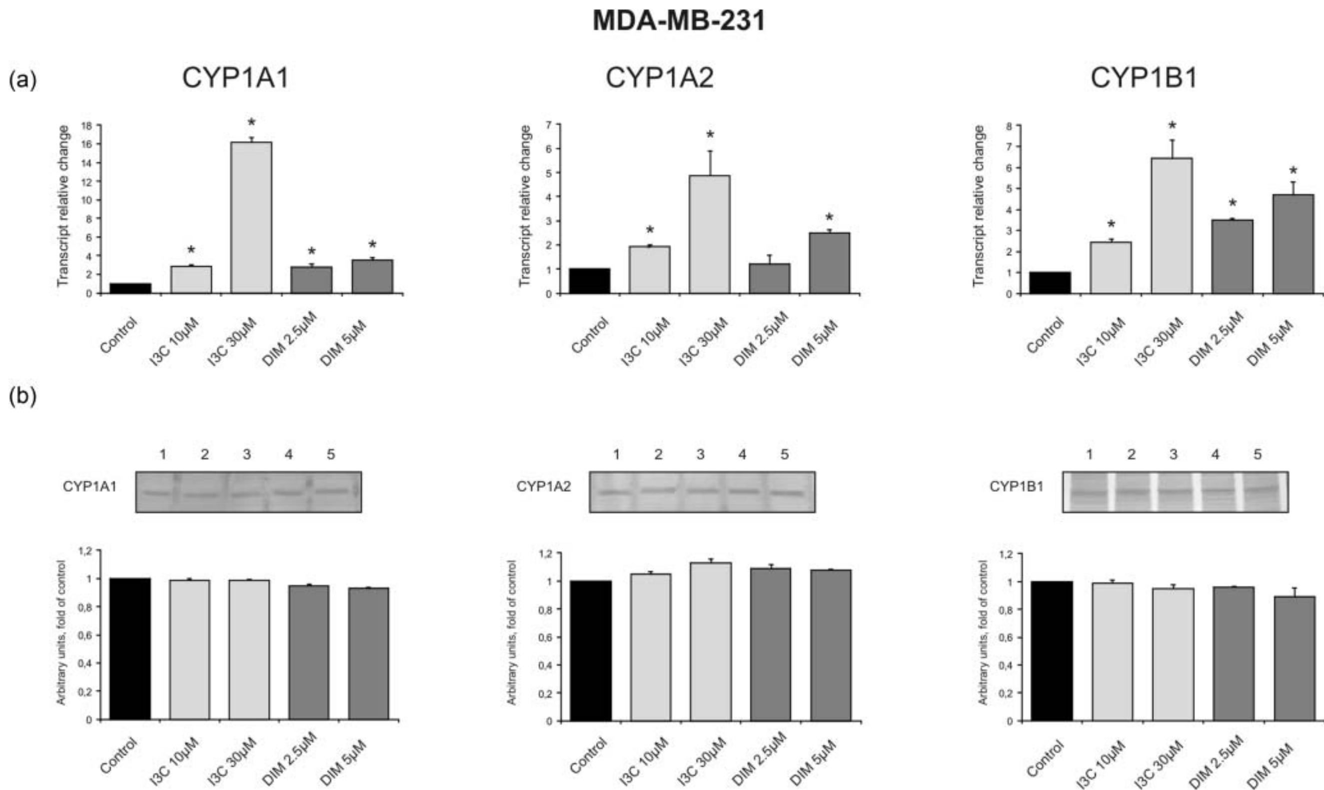

FIG. 4. The effect of 72-h incubation with indoles on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MDA-MB-231 cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); I3C 10 μM (lane 2); I3C 30 μM (lane 3); DIM 2.5 μM (lane 4); DIM 5 μM (lane 5). I3C = indole-3-carbinol; DIM = 3,3′-diindolylmethane.

TABLE 1
Ratio of CYP1A1 and CYP1A2 to CYP1B1 mRNA expression in cabbage juices- and indoles-treated MCF7, MDA-MB-231, and MCF10A cells

	MCF7			MDA-MB-231			MCF10A		
Treatment	Dose	Ratio CYP1A1/1B1	Ratio CYP1A2/1B1	Dose	Ratio CYP1A1/1B1	Ratio CYP1A2/1B1	Dose	Ratio CYP1A1/1B1	Ratio CYP1A2/1B1
SI	10 mL/L	10.36	4.44	2.5 mL/L	0.67	0.86	10 mL/L	3.23	1.17
SI	25 mL/L	4.44	3.29	5 mL/L	0.40	0.45	25 mL/L	4.65	0.54
RI	10 mL/L	1.99	3.06	2.5 mL/L	0.36	2.12	10 mL/L	1.77	1.17
RI	25 mL/L	1.22	1.56	5 mL/L	1.00	0.63	25 mL/L	1.01	0.48
SO	10 mL/L	2.83	4.81	5 mL/L	1.39	0.50	10 mL/L	7.88	9.14
SO	25 mL/L	10.60	1.79	10 mL/L	0.86	0.17	25 mL/L	8.67	3.81
RO	10 mL/L	2.07	1.36	5 mL/L	0.81	0.48	10 mL/L	2.01	0.27
RO	25 mL/L	3.22	1.74	10 mL/L	0.58	0.26	25 mL/L	1.32	0.30
I3C	30 μM	4.56	2.09	10 μM	1.18	0.80	10 μM	2.41	0.51
I3C	50 μM	5.79	1.11	30 μM	2.52	0.76	30 μM	2.89	0.59
DIM	5 μM	2.20	0.54	2.5 μM	0.79	0.34	5 μM	2.21	0.77
DIM	10 μM	1.79	0.20	5 μM	0.75	0.53	10 μM	3.11	0.64

SI = sauerkraut juice from industrial farming; RI = raw juice from industrial farming; SO = sauerkraut juice from organic farming; RO = raw juice from organic farming; I3C = indole-3-carbinol; DIM = 3,3′-diindolylmethane.

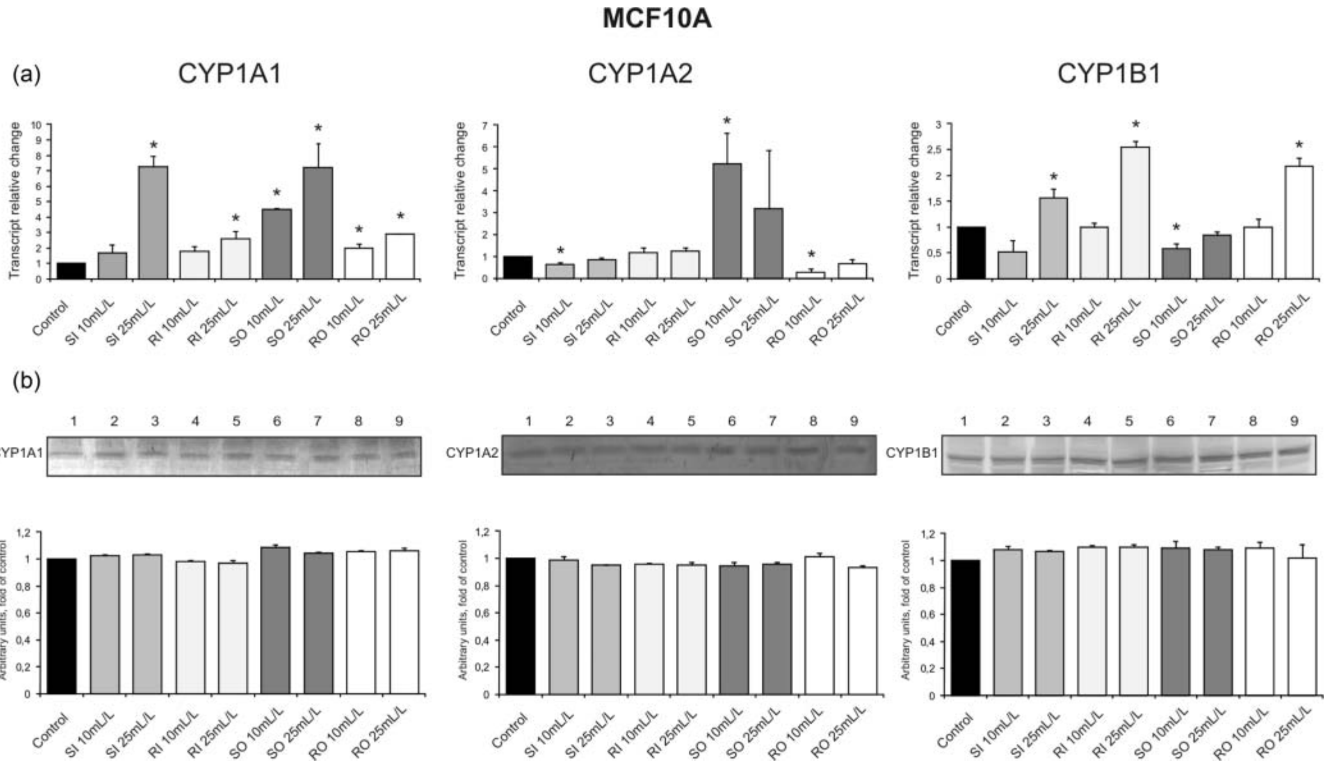

FIG. 5. The effect of 72-h incubation with cabbage juices on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MCF10A cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); SI 10 mL/L (lane 2); SI 25 mL/L (lane 3); RI 10 mL/L (lane 4); RI 25 mL/L (lane 5); SO 10 mL/L (lane 6); SO 25 mL/L (lane 7); RO 10 mL/L (lane 8); RO 25 mL/L (lane 9). SI = sauerkraut juice from industrial farming; RI = raw juice from industrial farming; SO = sauerkraut juice from organic farming; RO = raw juice from organic farming.

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sauerkraut juices in all doses resulted in the ratio of CYP1A1 to CYP1B1 greater than 1 (1.22 to 10.6 in MCF7; 1.32 to 8.67 in MCF10A). The ratios of CYP1A2 to CYP1B1 were lower in MCF7 but also above 1. In MDA-MB-231, however, the ratios in most cases were lower than 1, indicating preferential induction of CYP1B1 in these cells.

DISCUSSION

Cruciferous vegetables (*Brassicaceae*) are rich in GLS, which undergo hydrolysis to indoles (e.g., I3C and DIM), believed to be responsible for the chemopreventive properties of this food group. *Brassicaceae* representatives common in Central and Eastern European diet are raw or cooked cabbage and sauerkraut. Epidemiological migrant studies have shown that consumption of these food products during adolescence was associated with a 72% reduced risk of breast cancer (13). Estrogens, which are considered a major risk factor for breast cancer, are metabolized by the combined action of phase I and II enzymes (7). The phase I enzymes, CYP1A1, CYP1A2, and CYP1B1, catalyze oxidative metabolism of estrogens, leading to the formation of non/less harmful metabolites or DNA-damaging intermediates. Induction of estrogen metabolism via CYP1A1 and CYP1A2 usually results in reduction of carcinogenic estrogen level in breast cells, whereas the increased level of CYP1B1 contributes to higher amounts of estrogen reactive metabolites able to initiate tumorigenesis. The expression of these enzymes is controlled by the aryl hydrocarbon receptor (AhR). This ligand-activated protein affects estrogen action in two distinct pathways. In one pathway, AhR acts as a transcription factor that induces the expression of the CYP1 family of genes; in the other one, AhR initiates the degradation of the estrogen receptor and suppresses estrogen signaling (19). Thus different ligands may preferentially activate one of these pathways. Moreover, their effects may depend on breast cells' ER status.

In this study, two breast cancer cell lines differing in ER status, ER-positive MCF7 and ER-negative MDA-MB-231, along with immortalized breast nontumorigenic MCF10A cells, were used to evaluate the effect of juices obtained from cabbage of different origins and the effect of indoles, I3C, or DIM on the expression profile of CYP1A1, CYP1A2, and CYP1B1.

In accordance with the studies of other authors (10,20–22), constitutive expression of these P450 isoforms was detected in all tested cell lines, but the level of mRNA transcript of specific P450 was slightly different in comparison with those of the

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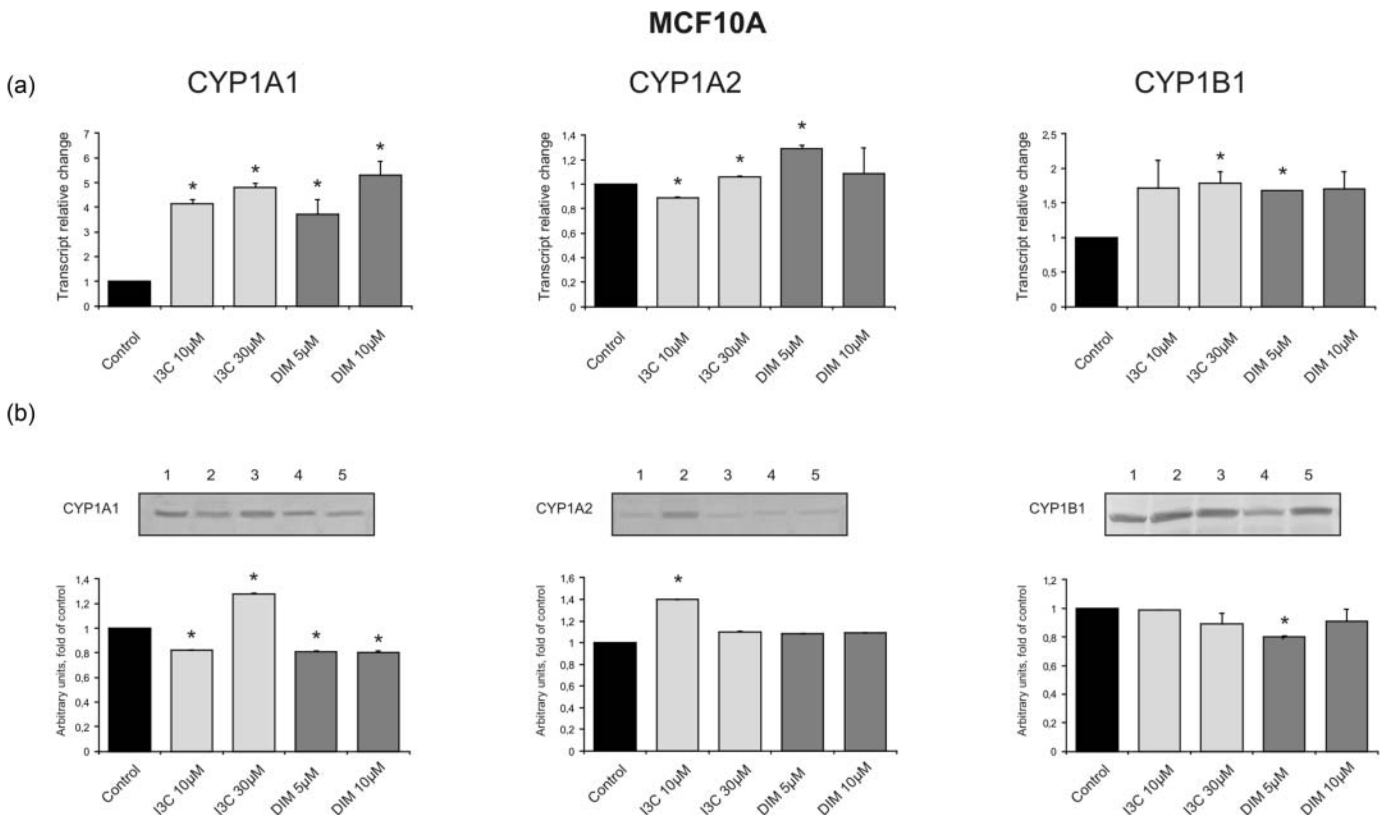

FIG. 6. The effect of 72-h incubation with indoles on the level of the CYP1A1, CYP1A2, and CYP1B1 transcript (a) and protein (b) in MCF10A cells. The values were calculated as a relative change in transcript or protein level in comparison to control cells (expression equals 1). The mean values ± SEM from 3 independent experiments run in duplicate (protein) or triplicate (mRNA) are presented. *Mean values were significantly different from the control group ($P < 0.05$). (b) Western blot analysis—representative blot is shown: control (lane 1); I3C 10 μM (lane 2); I3C 30 μM (lane 3); DIM 5 μM (lane 4); DIM 10 μM (lane 5). I3C = indole-3-carbinol; DIM = 3,3′-diindolylmethane.

reference genes. In particular, the relative level of CYP1B1 mRNA was higher in tumorigenic MCF7 cells (1.133) and MDA-MB-231 cells (1.135) than in nontumorigenic MCF10A cells (0.9584). This observation confirms the reports of the other investigators (23–25), as well as the possible role of CYP1B1 in breast cancer etiology (26).

Interestingly, our study showed also that the treatment with cabbage juices or indoles affected the expression of CYP1 family genes in the cell-type dependent manner. Although the induction of all tested P450 isoforms, particularly on mRNA level, was found in all cell lines, the ratio (Table 1) of CYP1A1 to CYP1B1 was 1.2- to 10.6-fold in favor to CYP1A1 in MCF-7 and MCF10A cells. Increased levels of CYP1A2 in comparison with CYP1B1 were also observed in MCF7 cells. In contrast to these noninvasive cells, in MDA-MB-231 cells CYP1B1 was preferentially induced. Thus it can be assumed that in nontumorigenic MCF10A and noninvasive MCF7 cells, cabbage juices may shift estrogen metabolism to production of 2-hydroxyestradiol and reduce the level of 4-hydroxyestradiol, thereby decreasing the opportunity of genotoxic insult by this highly reactive estrogen metabolite. In contrast, in more aggressive/invasive and ER-negative MDA-MB-231 cells cabbage juices may increase the production of DNA-damaging estradiol metabolites.

The results of the present study showed also that the most marked effect of cabbage juices as well as indoles, their pure active components, on cytochrome P450 isoforms was observed on the mRNA level. The protein levels in most cases were unchanged, slightly increased, or even decreased. The lack of correlation between the CYPs transcripts and protein levels was observed also by the other authors (26,27). The difference between mRNA levels and the corresponding protein levels may indicate that many of mRNA molecules do not reach the translational machinery, probably because the translation mechanism is saturated in the conditions of enhanced transcription. For example in the studies of Oleaga et al. (27), cocoa extract caused 32-fold increase in CYP1A1 mRNA, but only 1.2-fold increase in the protein level. On the other hand, it was suggested that a certain threshold level of mRNA must be achieved before the protein can be translated, or cell-specific posttranscriptional modifications including proteolytic degradation can modulate CYPs protein levels (21,28).

It is also worth noting that among cabbage juices, sauerkraut was the most effective modulator of the expression of

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cytochrome P450 among the cabbage juices tested. In particular, this effect was clearly visible in relation to CYP1A1 in MCF7 cells. The CYP1A1 expression was increased after treatment with sauerkraut juice obtained from both sources: the industrial and organic farms. The sauerkraut is the product of lactic acid fermentation of shredded and salted white cabbage. During shredding, glucobrassicin, the most commonly studied GLS, is transformed into I3C upon the action of myrosinase. During fermentation, as pH decreases, this indole reacts nonenzymatically with L-ascorbic acid to yield ascorbinogen (ABG), which is the dominant end product of indole GLS in sauerkraut (29,30). Although the effects of I3C have been extensively studied, only few experiments focusing on the anticarcinogenic effects of ABG have been published. The data provided by Stephensen et al. (31) and Kravchenko et al. (32) have shown that ABG itself or in combination with the other indoles (I3C and sulfraphane) modulates the enzymes involved in xenobiotic metabolism inducing CYP1A1 family. The interaction of ABG with AhR was suggested (32) as the mechanism of induction. The present study, showing the induction of CYP1 genes expression by sauerkraut, seems to confirm a possible role of ABG in this process.

As expected, indoles I3C and DIM induced to a high degree the expression of CYP1A1 and CYP1B1 and to less extent that of CYP1A2, in all cell lines tested. However, similarly to the cabbage juices the ratio of CYP1A1 to CYP1B1 transcript was higher in MCF7 and MCF10A cells than that found in MDA-MB-231 cells. The effect of DIM in MCF7 and MDA-MB-231 cells was less pronounced than that of I3C. One reason for this difference might be the lower dose of DIM used in this study. This dose corresponds however to the level achieved in plasma following consumption of a serving of cruciferous vegetables (33,34) and reflects the higher cytotoxicity of this compound in comparison with I3C shown in our previous studies (16). Moreover, in more invasive MDA-MB-231 cells representing the later stages of breast cancer development DIM preferentially induced CYP1B1, which may promote formation of reactive estrogen metabolites. Since similar effect was observed as result of cabbage juices treatment, it can be assumed that DIM might be responsible for CYP1 genes expression in this cell line. In less aggressive MCF7 and nontumorigenic MCF10A cells, I3C may be a more important cabbage component for P450 induction, particularly CYP1A1.

However, although it is generally accepted that the chemopreventive activity of GLS such as glucobrassicin is exclusively mediated by their degradation products, it has also been shown that intact GLS can modulate the activity of hepatic carcinogen-metabolizing enzymes (35). Thus it is possible that the effect of cabbage on CYP1 genes expression may result from combined action of both intact GLS and the products of their degradation.

The induction of CYP1A1 expression as a result of cabbage juices and indoles treatment of ER-positive and immortalized nontumorigenic cells may be considered as the mechanism of chemoprevention. However, such suggestion needs further studies to confirm such mechanism since the changes in mRNA transcript were not accompanied by similar changes in protein levels. Its induction in ER-negative cells may potentially increase sensitivity of these cells for conventional hormone therapy via increasing the ER level. This assumption is based on the fact that upregulation of CYP1A1 expression may increase the expression of ER (36).

In summary, the modulation of CYP1 genes expression on incubation with cabbage juices may partly explain the chemopreventive activity of cabbage products observed in breast cancer epidemiological studies. The CYP1 expression profile observed in this study indicates that by affecting hormonal system, cabbage may prevent breast cancer development. The exact mechanism of their involvement in estrogen metabolism, including binding to AhR requires further studies that are now under way. As estrogen and CYP1B1 can contribute also to development of other types of cancer (e.g., head and neck cancer) (37), the observations from this study might be important for the prevention of other cancers besides the breast.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Higher Education of Poland, grant N N405 3390 33.

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