Differential Effects of Vegetable-Derived Indoles on the Induction of Quinone Reductase in Hepatoma Cells

Yue-Hwa CHEN* and Darlene YANG

Graduate Institute of Nutrition and Health Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan 110, R.O.C.

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Summary The increased expression of quinone reductase (QR) has been associated with anticarcinogenic processes. The aim of this study was to explore the roles of the cruciferous vegetable-derived indoles, indole-3-carbinol (I3C) and indolo[3,2-b]carbazole (ICZ), on the regulation of QR in both murine (Hepa-1) and human (HepG2) hepatoma cells. The results indicate that ICZ enhanced QR activity in both Hepa-1 and HepG2 cells, whereas its parent compound, I3C, had no significant effect on the induction of QR. Moreover, the ICZ-induced QR activity showed a higher response and expressed a more-significant dose-response in Hepa-1 cells. QR mRNA expression as analyzed by RT-PCR demonstrated a pattern similar to that of the enzyme activity. In conclusion, I3C did not show an enhancement effect on QR activity, but its acidic derivative, ICZ, increased the expression of QR mRNA, which then caused the augmentation of QR activity in Hepa-1 and HepG2 cells.

Key Words quinone reductase, cruciferous vegetable, dietary indole, indolo[3,2-b]carbazole, hepatoma cells

Vegetables and fruits not only contain essential nutrients to maintain life, but also a variety of phytochemicals, non-nutritive compounds that possess health-promoting activities, that provide protection from many diseases including cancer (1-3). A number of phytochemicals have been identified as possessing such activity, including indoles in cruciferous vegetables (4, 5). Several epidemiological studies have shown that cruciferous vegetables, cabbage, Napa cabbage, broccoli, and cauliflower, play a protective role against various kinds of cancers (6, 7). A number of animal experiments have also indicated that indoles derived from these vegetables play important roles in cancer prevention (8-10).

The modification of xenobiotic-metabolizing enzymes by dietary chemopreventive compounds is thought to be closely associated with their anticarcinogenic effect (11, 12). NAD(P)H:quinone reductase or QR (EC 1.6.99.2), also known as DT diaphorase, is a phase I enzyme mainly found in the liver and other tissues. It catalyzes two-electron reduction of quinone and quinoid compounds, so it is important in protecting cells against mutagenicity and carcinogenicity generated from free radicals and toxic oxygen metabolites produced from the one-electron reduction reaction via cytochrome P450s (CYP) and other enzymes (13, 14). QR is induced in response to various xenobiotics, including planar polycyclic and heterocyclic aromatic hydrocarbons such as β-naphthoflavone (BNF) and 3-methylcholanthrene (3MC), phenolic antioxidants such as t-butylhydroquinone (BHQ), and 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD). Evidence indicates that the induction of QR by these compounds is transcriptionally regulated (14-17).

Indole-3-carbinol (I3C) is one of the indolic compounds present in large quantities in many cruciferous vegetables. Under acidic conditions, I3C can be further converted to other oligomeric products including indolo[3,2-b]carbazole (ICZ) (18, 19). A number of studies have demonstrated that cruciferous vegetables and I3C can modulate QR activity in animals and cultured cells (20-26), but it is not clear whether I3C per se or its derivatives are responsible for such induction. Since induction of the QR enzyme may be regarded as a detoxifying or a protective process (27, 28), and because hepatoma cells are responsive to various xenobiotics and express high levels of xenobiotic-metabolizing enzymes, the present study investigated the effects of I3C and ICZ on the expression of QR in both murine and human hepatoma cells.

MATERIALS AND METHODS

Chemicals and biochemicals. ICZ was kindly provided by Dr. Leonard F. Bjeldanes of the University of California, Berkeley. I3C, flavine-adenine dinucleotide (FAD), glucose 6-phosphate (G6P), G6P dehydrogenase, digitonin, menadione, nicotinamide adenine dinucleotide phosphate (NADP), crystal violet, diethyl pyrocarbonate (DEPC), phenol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical (St. Louis, MO, USA). β-Naphthoflavone (BNF) was from Aldrich (Milwaukee, WI, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum, and trypsin were purchased from GIBCO.

* To whom correspondence should be addressed.
E-mail: yuehwa@tmu.edu.tw

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were used for mouse Hepa-1 cells (32), QR primers 5'-TAATTGTAAGCAAACTCTCCTATG-3' and 5'-CTAGGCTCTTCTCCA-3' were used as the internal control (32). The reaction mixture (50 µL) for PCR contained 1 µL of template DNA, 0.2 µM each of the 5' and 3' primers, 0.2 mM dNTPs, 1.5 mM MgSO₄, 10×PCR buffer, and 2.5 units of PLATINUM Taq polymerase (GIBCO) mixed with the cDNA products. The amplification procedure involved heating to 94°C for 1 min with immediate cycling 27 times (denaturation at 94°C for 30 s; annealing at 55°C for 45 s; and elongation at 72°C for 60 s). This was followed by heating at 72°C for 5 min to stop the reaction. Five microliters of the PCR reaction products was then electrophoresed on a 2.5% DNA agarose gel and visualized by ethidium bromide staining.

Statistical analysis. Values are expressed as the mean±SD. Student's t-test was used to determine statistical differences between groups using SAS software Version 6.12 (SAS Institute, Cary, NC, USA). The significance of mean differences was based on a p value of <0.05.

RESULTS

To examine the effects of I3C on QR activity in hepatoma cells, cells were treated with different concentrations of I3C, and QR activity was determined after 16, 24, and 48 h of treatment. The results indicated that I3C, at concentrations ranging from 10⁻¹⁰ to 10⁻³ M, did not affect QR activity in either Hepa-1 (Fig. 1) or HepG2 cells (data not shown). On the other hand, ICZ, an acidic derivative of I3C, showed induction activity. As shown in Fig. 2, ICZ enhanced QR activity in a concentration-dependent manner in Hepa-1 cells.
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Fig. 2. Effect of ICZ on the induction of OR activity in Hepa-1 cells. Cells were treated with various concentrations of ICZ as indicated or with BNF (2 μM) for 24 h. OR activity was then determined as described in Materials and Methods. Columns represent the mean±SD from eight measurements. *p<0.05 as compared to the DMSO control.

Fig. 3. Effect of ICZ on the induction of QR activity in Hepa-1 cells. Cells were treated with various concentrations of ICZ as indicated or with BNF (2 μM) for 24 h. QR activity was then determined as described in Materials and Methods. Columns represent the mean±SD from eight measurements. *p<0.05 as compared to the DMSO control.

after 24 h of treatment, and the activity was 1.7-fold that of the DMSO control at a concentration as low as 3.125 nM. In the meantime, we also observed that the maximal ICZ concentration used (3.125 μM) caused a 2.8-fold induction over that of the DMSO control, but it was not as potent as the BNF, which induced a fourfold increase in QR after 24 h of treatment. Other than Hepa-1 cells, ICZ also enhanced QR activity in HepG2 cells (Fig. 3), but it induced only a 1.5-fold increase in QR at the highest concentration used (3.125 μM), indicating that the ICZ-induced QR induction in HepG2 cells was not as potent as that in Hepa-1 cells.

Since ICZ can enhance QR activity in hepatoma cells, and because the expression of QR is transcriptionally regulated, RT-PCR was performed to examine whether or not the induced QR activity was due to the increased expression of QR mRNA. Parallel to the enzyme activity, the level of QR mRNA increased with increasing concentrations of ICZ after 24 h of treatment in Hepa-1 cells. As shown in Fig. 4A, the relative location of the amplified QR cDNAs after agarose electrophoresis in Hepa-1 cells was similar to that in a study previously described (32). In addition, the amplified QR cDNA (379 bp) in HepG2 cells did not show a significant concentration-dependent effect (Fig. 4B and 4C) with ICZ treatment.

DISCUSSION

In the present study, we observed that the cruciferous vegetable derivative, ICZ, increased the expression of phase I enzyme QR activity in both murine and human hepatoma cells, but its parent compound, I3C, showed no such effect. I3C is one of the indole derivatives present in large concentrations in cruciferous vegetables. Under acidic conditions, I3C may be converted to other derivatives. Grose and Bjeldanes reported that several products, including ICZ, 3,3′-diindolylmethane (3,3′-DIM), and several polymers, are formed when I3C is treated with 0.1 N HCl in vitro (19). In vivo, ICZ can also be formed in the stomach when animals are orally fed I3C (18). It has been shown that the oral administration of I3C may increase hepatic QR activity in animals (20–23), whereas no such induction was observed when I3C was administered i.p. (34). These results, together with our observations, suggest that the QR induction activity evoked by the ingestion of cruciferous vegetables containing I3C is not due to I3C per se, but is due to, at least partially, the production of ICZ in the stomach. Since other components in cruciferous vegetables and other I3C derivatives, such as crambe, isothiocyanates, sulforaphane, and 3,3′-DIM, have also been reported to increase QR activity in rats and in primary hepatocytes (21, 24, 26, 35, 36), they may also play important roles in the induction of QR after the dietary administration of cruciferous vegetables or I3C.

Consistent with previous studies, the induction of QR by ICZ acts through the increased expression of QR mRNA. Several DNA sequences on the qr gene have been identified to be involved in the regulation of QR expression, including the xenobiotic responsive element (XRE) and antioxidant responsive element (ARE). The XRE sequence is identical to that found in the cyp1a1 gene and gst gene; its action is dependent upon the binding of the ligand to the cytosolic aryl hydrocarbon (Ah) receptor. After binding to the inducers, the Ah receptor translocates to the nucleus and binds to the Ah receptor nuclear translocator (Arnt) protein. In the nucleus, the Ah receptor-Arnt protein complex interacts with XRE upstream of the Ah receptor-responsive genes, including the qr, gst, and cyp1a1 genes, and thus enhances transcription of the corresponding genes. It
has been reported that ICZ possesses high binding affinity to the Ah receptor (37), and that the ICZ-activated receptor complex can interact with XRE in Hepa-1 cells (38). Therefore, it is likely that ICZ activates the \( qr \) gene via the Ah receptor-XRE pathway.

QR is also inducible by various phenolic antioxidants via ARE without requiring the Ah receptor. ARE is responsive to phenolic antioxidants such as BHQ and certain metabolizable xenobiotics, including BNF, but it is not responsive to TCDD (39). Thus, metabolizable xenobiotics such as BNF may transcriptionally activate the \( qr \) gene through the XRE as well as the ARE, whereas
non- or poorly metabolizable compounds such as TCDD activate the \( q_r \) gene only through the Ah receptor-XRE pathway. Apparently, ICZ can be rapidly metabolized in cells (38). In addition, the potential roles of cruciferous vegetables and their indolic derivatives as antioxidants have been reported (40–42). The possibility that ICZ acts through the ARE sequence to activate the expression of the OR cannot be ruled out, but this needs to be investigated further.

In the present study, we demonstrate that ICZ has a higher and more-sensitive QR inducibility in Hepa-1 than in HepG2 cells. The Hepa-1 cell line is derived from murine hepatoma cells that express high levels of xenobiotic-metabolizing enzymes, such as CYP1A1. The HepG2 cell line is derived from human hepatoblastoma cells, and it has also been shown to express various xenobiotic-metabolizing enzymes. Kikuchi et al. reported that benzimidazole compounds show different inducibilities of CYP1A1 between Hepa-1 and HepG2 cells. They demonstrated that these compounds increase the expression of CYP1A1 mRNA in HepG2 cells, but no such induction was shown in Hepa-1 cells. The authors suggest that a cellular factor in HepG2 cells plays an important role in activation of the Ah receptor (43). Therefore, different cellular factor(s) in Hepa-1 and HepG2 cells may also contribute to the variation in QR inducibility evoked by ICZ.

Modification of xenobiotic-metabolizing enzymes by dietary chemopreventive compounds is closely associated with their anticarcinogenic effects (11, 12). Cruciferous vegetables and their derivatives have been shown to induce both phase I and phase II xenobiotic-metabolizing enzymes, including CYP1A1, QR, and GST (20, 21, 44). These compounds have also been reported to reduce levels of carcinogen-DNA adducts (45) and induce cell cycle arrest and apoptosis in cultured cells (46). Because epidemiological studies have also indicated the anticarcinogenic properties of cruciferous vegetables (6, 7), the overall outcome of cruciferous vegetable consumption may therefore be more toward protection of the cells from carcinogenic pathways. Two major classes of phytochemicals, indoles and isothiocyanates, have been identified in cruciferous vegetables, and both have been shown to involve protective effects. Among these, the indolic derivative ICZ induces QR activity at a concentration as low as 3.125 nm in Hepa-1 cells, and induces CYP1A1 activity at 67.5 nm (38). One serving of Brussels sprouts (100 g) would provide a dose of 1–5 nmol of ICZ (47) and produce 0.2–1 nm of plasma concentration in a 70 kg person based on blood volume in the adult human that comprises 7% of body weight. Hence, the effect of the ordinary consumption of Brussels sprouts seems to be able to provide effective concentrations of ICZ in the blood.

In conclusion, we have demonstrated that the ICZ derived from cruciferous vegetables does not induce QR activity in hepatoma cells. However, its acidic derivative, ICZ, enhances the expression of QR mRNA, thus increasing QR activity in both murine and human hepatoma cells, and such induction may explain, at least in part, the anticarcinogenic effect of cruciferous vegetables.

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