Oltipraz Inhibits 3-Methylcholanthrene Induction of CYP1A1 by CCAAT/Enhancer-binding Protein Activation*

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Oltipraz, a cancer chemopreventive agent, induces CYP1A1 to a certain extent by transactivation of the gene via the Ah receptor (AhR)- xenobiotic response element (XRE) pathway. Previously, we showed that oltipraz promoted CCAAT/enhancer binding proteinβ (C/EBPβ) activation, which leads to the induction of glutathione S-transferase. Given that oltipraz activates C/EBPβ for gene transactivation and that the putative C/EBP binding site is located in the CYP1A1 promoter region, this study investigated the effect of oltipraz on CYP1A1 induction by 3-methylcholanthrene (3-MC). 3-MC induced CYP1A1 in H4IIE cells in a time- and concentration-dependent manner. Gel shift analysis showed that 3-MC increased the band intensity of protein binding to the XRE. Immunocompetition analysis verified the specificity of AhR-XRE binding. Oltipraz (30 μM) induced CYP1A1 and the CYP1A1 promoter-luciferase gene and increased AhR DNA binding activity, which was 10–20% of those in 3-MC (100 nM)-treated cells. However, AhR-XRE binding was not increased after 10 μM oltipraz treatment. Oltipraz (10 μM) significantly inhibited CYP1A1 and CYP1A1-luciferase gene induction by 3-MC with no increase in AhR DNA binding. Oltipraz enhanced protein binding to the C/EBP binding site in the gene promoter and the binding complex comprised of C/EBPβ and partly C/EBPα. Overexpression of dominant-negative mutant C/EBPβ significantly abolished the ability of oltipraz to suppress 3-MC-inducible CYP1A1 and the CYP1A1 reporter gene expression. Consistently, C/EBPβ overexpression blocked CYP1A1 reporter gene induction by 3-MC. These results provide evidence that oltipraz suppresses 3-MC induction of CYP1A1 gene expression and that activation of C/EBPβ by oltipraz contributes to suppression of 3-MC-inducible AhR-mediated CYP1A1 expression.

Polycyclic aromatic hydrocarbons (PAHs) induce a battery of drug-metabolizing enzymes in hepatic and extrahepatic tissues. Among xenobiotic-metabolizing enzymes, CYP1A1, which possesses distinct substrate specificity, bioactivates a number of procarcinogens and toxicants (2). Exposure of animals or cells to PAHs leads to CYP1A1 induction. The substrates and inducers of CYP1A1 include 3-methylcholanthrene (3-MC) (3), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (4), benzo(a)pyrene (5), and nitropyrene (6). PAHs regulate gene expression via a specific aryl hydrocarbon receptor (AhR). The heterodimeric AhR and aryl hydrocarbon receptor nuclear translocator (ARNT) complex bind to the XREs located upstream of target genes and promote transactivation (7, 8). Epidemiology studies show that individuals with a high level of CYP1A1 gene expression were at greater risk for cancer incidence (9).

Oltipraz, a synthetic derivative of 1,2-dithiole-3-thione, serves as a cancer chemopreventive agent by inducing phase II detoxifying enzymes (10–12). The induction of glutathione S-transferases (GSTs) by oltipraz is associated with cancer chemopreventive and cytoprotective effects (14–17). The members of the CCAAT/ enhancer-binding protein (C/EBP) family, which have roles in cell proliferation and differentiation, regulate the expression of tissue-specific genes, including C/EBPβ and C/EBPα (18, 19). We recently found that activation of C/EBPβ and its binding to the C/EBP-response element play a critical role in the induction of the GSTA2 gene (11, 20). Oltipraz and flavonoid compounds promote phosphoinositide 3-kinase-mediated nuclear translocation of C/EBPβ, which leads to the induction of the GST gene by activating binding to the C/EBP-response element in the GSTA2 gene. Signals activated by oxidative stress stimulate transduction of NF-E2-related factor-2 activity and activation of ARE (16, 21). Thus, the pathways involving both C/EBP and NF-E2-related factor-2 are essential and distinct for the phase II enzyme induction.

Oltipraz affects activities and the expression of cytchrome P450s. This agent inhibited CYP1A1 activity in vivo and in vitro (22). Other studies showed that oltipraz increased the expression of CYP1A1 in the rat liver, kidney, and lung following its inhibition of metabolic activity (23–25). It has also been shown that oltipraz is an inducer of the XRE-containing 5′-flanking region of the CYP1A1 gene (25). The proteins that bind to the XRE in the gene promoter have been found to be relevant to a member of the C/EBP family of transcription factors. C/EBPα showed overlapping DNA binding specificity to that of the AhR (26). Other studies provided evidence that several common transcriptional factors bind to the AhRE motif of the murine Cyp1a1 gene, indicated by competition studies with an excess of AhRE3, mutated AhRE3, and C/EBPα oligonucleotides (27).

In view of the fact that oltipraz activates C/EBPβ for gene

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The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; 3-MC, 3-methylcholanthrene; AC/EBP, dominant-negative mutant C/EBP; AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; ARNT, aryl hydrocarbon receptor nuclear translocator; C/EBP, CCAAT/enhancer-binding protein; CYP1A1, cytochrome P450A1; MEM, minimal essential medium; XRE, xenobiotic response element; GST, glutathione S-transferase.
Fig. 1. The effects of 3-MC on the expression of CYP1A1 and the activation of AhR. A, immunoblot analysis of CYP1A1 in cells treated with various concentrations of 3-MC (1 nM to 10 μM, 24 h). The relative CYP1A1 levels were assessed by scanning densitometry of immunoblots. Each lane was loaded with 10 μg of microsomal protein. Data represent the mean ± S.D. of three separate experiments (the level of CYP1A1 in cells treated with 100 nM 3-MC is 100%). B, immunoblot analyses of CYP1A1 in cells treated with 3-MC for 3–48 h. Representative immunoblots show the levels of CYP1A1 (and CYP1A2) protein(s) in H4IIE cells treated with 100 nM 3-MC for 3–48 h. Each lane was loaded with 10 or 20 μg of microsomal protein (the level of CYP1A1 in cells treated with 3-MC for 12 h is 100%). C, gel shift analysis of AhR binding to the XRE. Nuclear extracts were prepared from H4IIE cells incubated with 100 nM 3-MC for 3–12 h and subjected to gel shift analysis. All lanes contained 15 μg of nuclear extracts and 5 ng of radiolabeled XRE oligonucleotide. For immunoinhibition, the nuclear extract obtained from cells treated with 3-MC for 6 h was incubated with an anti-AhR antibody for 1 h. The immunodepleted extract was mixed with labeled probe. Competition experiments using 20-fold excess XRE or SP-1 oligonucleotide confirmed the specificity of protein binding to the XRE. Arrowhead indicates DNA bound with AhR.
transactivation and that the C/EBP protein is involved in the formation of the transcription complexes binding to the XRE, we investigated whether oltipraz alters AhR-mediated CYP1A1 induction by 3-MC and whether activating C/EBP binding to the putative C/EBP binding site in the CYP1A1 gene promoter, inhibits AhR-bound XRE-mediated transcription of the gene.

We now report that oltipraz suppresses 3-MC-inducible CYP1A1 expression and that activating C/EBPβ binding to the putative C/EBP binding site in the CYP1A1 gene promoter.
Inhibition of PAH Induction of CYP1A1 by Oltipraz

Fig. 3. Induction of CYP1A1 promoter-luciferase activity by 3-MC or oltipraz. A, scheme showing the XRE and NRE sites present in the chimeric gene construct pGL-CYP1A1-1195 that contained the promoter region of CYP1A1 and the coding region of luciferase. XRE, negative regulatory element. B, luciferase activity was measured in H4IIE cells transiently transfected with pGL-CYP1A1-1195. Dual luciferase reporter assay was performed with the lysates obtained from cells co-transfected with the CYP1A1-luciferase gene construct (firefly luciferase) and pRL-SV (Renilla luciferase) (a ratio of 200:1) after exposure to 3-MC (100 nM) or oltipraz (10 \( \mu \)M) for 18 h. Activation of the reporter gene was calculated as a relative change to the negative regulatory element.

which is promoted by oltipraz, is responsible for the inhibition of 3-MC-inducible AhR-mediated CYP1A1 expression.

EXPERIMENTAL PROCEDURES

Materials—\( ^{32} \)P-ATP (3000 mCi/mmol) was purchased from PerkinElmer Life Sciences (Arlington Heights, IL). Anti-CYP1A1/2 antibody was supplied from Oxford Biomedical Research, Inc. (Oxford, MI). Horseradish peroxidase-conjugated goat anti-mouse IgG was obtained from Zymed Laboratories Inc. laboratory (San Francisco, CA). Anti-AhR, anti-C/EBP\( \alpha \), anti-C/EBP\( \beta \), and anti-C/EBP\( \delta \) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). These antibodies specifically recognized their respective transcription factors without any cross-reactivity. Oltipraz was kindly provided by Sigma Chemical.

Cell Culture—H4IIE cells, a rat hepatocyte-derived cell line, were obtained from American Type Culture Collection and maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (FCS), 50 units/ml penicillin, and 50 \( \mu \)g/ml streptomycin at 37°C in a humidified atmosphere with 5% CO\(_2\).

Immunoblot Analysis—H4IIE cells were incubated with 1 \( \mu \)M to 10 \( \mu \)M 3-MC and/or 10–30 \( \mu \)M oltipraz for the indicated time period. After washing the cells twice with sterile phosphate-buffered saline, the cells were scraped and sonicated for disruption. Microsomal fractions were prepared by differential centrifugation. The microsomal fractions were stored at -70°C until use. SDS-polyacrylamide gel electrophoresis and immunoblot analyses were performed according to previously published procedures (28). Microsomal proteins were separated by 7.5% gel electrophoresis and electrophotographically transferred to nitrocellulose paper. The nitrocellulose paper was incubated with anti-CYP1A1/2 antibody, followed by incubation with horseradish peroxidase-conjugated secondary antibody, and developed using ECL\( \textregistered \) chemiluminescence detection kit (Amersham Biosciences, Buckinghamshire, UK). Equal loading of proteins was verified by Coomassie staining. Changes in the levels of CYP1A1 were determined via scanning densitometry. At least three separate experiments were performed with different lysates to confirm changes in the protein levels.

Preparation of Nuclear Extracts—Nuclear extracts were prepared essentially according to the previously published method (29). Briefly, the cells in dishes were washed with ice-cold phosphate-buffered saline. Cells were then scraped, transferred to microtubes, and allowed to swell after the addition of 100 \( \mu \)l of hypotonic buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.5% Nonidet P-40, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride. The lysates were incubated for 10 min in ice and centrifuged at 7200 \( \times \) g for 5 min at 4°C. Pellets containing crude nuclei were resuspended in 50 \( \mu \)l of extraction buffer containing 20 mM HEPES (pH 7.9), 400 mM NaCl, 1 mM EDTA, 10 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride and then incubated for 30 min in ice. The samples were centrifuged at 15,800 \( \times \) g for 10 min to obtain supernatants containing nuclear fractions. Nuclear fractions were stored at -70°C until use.

Gel Retardation Assay—Double-stranded DNA probes containing the consensus C/EBP binding oligonucleotide, the rat CYP1A1 XRE oligonucleotide, and the putative CYP1A1 C/EBP binding oligonucleotide were used for gel shift analyses after end-labeling of each probe with \( ^{32} \)P-ATP and T\(_{4}\) polyribonucleotide kinase. The sequences of C/EBP consensus, CYP1A1 XRE and putative CYP1A1 C/EBP binding oligonucleotides were 5'-TGAGTTGCCAGTACGTCA-3', 5'-GGAGTTGCCAGAAGGCGC-3' (30) and 5'-TGTAGCTTGCTAAGGCGT-3', respectively. The reaction mixtures included 4 \( \mu \)l of 5 \( \times \) binding buffer containing 20% glycerol, 5 mM MgCl\(_2\), 250 mM NaCl, 2.5 mM EDTA, 2.5 mM dithiothreitol, 0.25 mg/ml poly dI-dC, and 50 mM Tris-Cl (pH 7.5), 15 \( \mu \)g of nuclear extracts, and sterile water in a total volume of 20 \( \mu \)l. The reaction mixtures were preincubated for 10 min. DNA binding reactions were carried out at room temperature for 30 min after the addition of 1 \( \mu \)l of probe (10\(^{6}\) cpm). Specificity of binding was deter-
mined by competition experiments, which were carried out by adding a 20-fold excess of unlabeled XRE, C/EBP, or SP-1 oligonucleotide to the reaction mixture before the DNA binding reaction. For the supershift assay, antibodies (1 μg each) were added to the reaction mixture, and additionally incubated for 1 h at 25 °C. Samples were loaded onto 4% polyacrylamide gels at 100 V. The gels were removed, fixed, and dried, followed by autoradiography.

Plasmid Construction—Firefly luciferase reporter gene construct, pGL-CYP1A1-1195 was generated by ligation of the 1195-bp promoter region of the rat CYP1A1 gene with the coding region of luciferase. Briefly, rat genomic DNA was prepared by using Wizard® SV genomic DNA purification system (Promega, Madison, WI). The flanking region of the CYP1A1 gene was generated by PCR amplification using specific primers. The amplified fragment was inserted into pGEM-T vector (Promega, Madison, WI) and subcloned into the MuI/BglII sites of the pGL2 reporter plasmid (Promega, Madison, WI). C/EBP-specific dominant-negative expression (AC/EBP) plasmid was a gift from Dr. C. Vinson (National Institutes of Health, Bethesda, MD) (31). pCDNA-C/EBPβ, which encodes C/EBPβ, was produced by PCR amplification of the coding region of the C/EBPβ gene using rat genomic DNA as a template (32) and the amplified DNA was then cloned into pCDNA3.1(+) (Invitrogen, Carlsbad, CA). The DNA sequences of the pGL-CYP1A1-1195 and pCDNA-C/EBPβ constructs were verified by sequence analysis using an ABI7700 DNA cycle sequencer.

CYP1A1 Promoter Luciferase Assay—The CYP1A1 promoter-luciferase reporter gene assay was carried out according to previously published methods with some modifications (11). Briefly, cells (7 × 10^5 cells/well) were replated in 6-well plates overnight, serum-starved for 6 h, and transiently transfected with the CYP1A1 promoter-luciferase construct (1 μg each) and pRL-SV plasmid (5 ng each, Renilla luciferase expression for normalization) (Promega, Madison, WI) using LipofectAMINE Plus® Reagent for 3 h (Invitrogen, Carlsbad, CA). Transfected cells were incubated in the medium containing 1% fetal calf serum for 13 h, and then exposed to 30 nM 3-MC in the presence or absence of oltipraz (10 μM) for 18 h or 24 h. Firefly and Renilla luciferase activities in cell lysates were measured using a luminometer (Luminoskan® Plus, Thermo Labsystems, Helsinki, Finland). The relative luciferase activity was calculated by normalizing CYP1A1 promoter-driven firefly luciferase activity to that of Renilla luciferase. For some experiments, cells were co-transfected with pCMV-AC/EBP or...
RESULTS

CYP1A1 Induction by 3-MC—Western blot analysis was performed to confirm that 3-MC induced CYP1A1 in H4IIE cells. CYP1A1 was induced 24 h after 3-MC treatment at the concentration of 1 μM or above in a concentration-dependent manner (Fig. 1A). CYP1A1 induction plateaued at 1 μM. Because 10–100 nM 3-MC significantly induced CYP1A1, the concentrations were chosen in subsequent experiments. A time course study showed that 3-MC at the concentration of 100 nM induced CYP1A1 with the maximal induction being observed at 6 h or later (Fig. 1B).

We determined whether 3-MC induced ligand-activated AhR binding to the XRE. Gel shift analysis showed that 3-MC increased the band intensity of protein binding to the XRE consensous oligonucleotide (Fig. 1C). The band intensity of AhR binding to the XRE substantially increased from 3 to 6 h, followed by returning to control level at 12 h. The specificity of AhR binding to the XRE (6 h) was verified by immunocompetition analysis using an anti-AhR antibody (Fig. 1C). Competition experiments using a 20-fold excess of unlabeled XRE or SP-1 oligonucleotide confirmed the specificity of protein binding to the XRE (Fig. 1C).

Effect of Oltipraz on CYP1A1 Expression—Previous studies have shown that oltipraz (e.g. at 50–100 μM) induces CYP1A1 and activates transription of the CYP1A1 gene through the AhR-XRE pathway (25). We compared the extent of CYP1A1 induction by oltipraz with that by 3-MC. The relative CYP1A1 protein level in H4IIE cells treated with 30 μM oltipraz was ~25% of that induced by 100 nM 3-MC (Fig. 2A). Oltipraz at the concentration of 10 μM was minimally active. Next, we tested whether oltipraz was capable of activating AhR binding to the XRE. Gel shift assay showed that oltipraz (30 μM, 3 h) increased protein binding to the XRE, which was much weaker than that induced by 3-MC (100 nM, 3 h) (Fig. 2B). AhR binding activity to a radiolabeled XRE oligonucleotide maximally increased 3 h after treatment of H4IIE cells with 30 μM oltipraz and returned toward that of control at 6 h or later time point (Fig. 2B). Oltipraz at the concentration of 10 μM did not activate AhR binding to the XRE (Fig. 2B). An increase in the band intensity of protein DNA binding by 30 μM oltipraz (3 h) was specific to the XRE, as evidenced by competition experiments using a 20-fold excess unlabeled XRE or SP-1 oligonucleotide (Fig. 2B).

We then examined the effect of oltipraz on transcription activity of the CYP1A1 gene by using the CYP1A1 promoter reporter gene assay. H4IIE cells were transfected with a reporter vector pGL-CYP1A1-1195, which contained the luciferase structural gene and the -1.2-kb rat CYP1A1 promoter (Fig. 3A). Exposure of H4IIE cells, transiently transfected with the plasmid, to 10 or 30 μM oltipraz resulted in increases in luciferase activity, which was 15 and 25% of that induced by 100 nM 3-MC, respectively (Fig. 3B).

Inhibition of 3-MC-Inducible CYP1A1 Expression by Oltipraz—We previously showed that oltipraz promotes activation of C/EBPβ, which contributes to GSTA2 induction via activating C/EBPβ binding to the C/EBP binding site present within the XRE in the GSTA2 gene (11, 21). Because the putative C/EBP binding site is located in proximity to the XRE in the promoter region of the CYP1A1 gene, we were interested in whether oltipraz might affect 3-MC-inducible CYP1A1 expression via activation of C/EBPβ. We determined the extents of CYP1A1 induction in H4IIE cells treated with 10–100 nM 3-MC in combination with 10 μM oltipraz. Western blot analysis revealed that oltipraz (10 μM) significantly blocked the induction of CYP1A1 by 10 or 30 nM 3-MC, whereas CYP1A1 induction by 100 nM 3-MC was not significantly decreased by concomitant treatment with oltipraz (Fig. 4A). To verify the inhibition by oltipraz of 3-MC induction of CYP1A1, luciferase reporter gene analyses were performed. The luciferase induction by 3-MC (30 nM) in pGL-CYP1A1-1195-transfected cells was markedly decreased by 10 μM oltipraz treatment (Fig. 4B).

These results demonstrated that oltipraz was capable of suppressing 3-MC induction of the CYP1A1 gene.

Next, we assessed whether oltipraz altered the extent of ligand-activated AhR binding to the XRE by gel shift analysis. AhR binding to the XRE in cells treated with 30 nM 3-MC was completely abolished by concomitant treatment with oltipraz (10 μM, 6 h) (Fig. 5).

Activation of the C/EBP Binding Site in the CYP1A1 Promoter by Oltipraz—To test whether C/EBPβ was involved in alteration of CYP1A1 gene expression, gel shift analyses were...
conducted with the putative C/EBP binding site present in the CYP1A1 gene promoter (Fig. 6A), with nuclear fractions. Protein binding to the putative C/EBP binding site was increased 6 h after oltipraz treatment (30 μM), which is in parallel with the previous observation (11). Competition experiments using excess amounts of unlabeled C/EBP or SP-1 binding oligonucleotides (20-fold) confirmed the specificity of C/EBP DNA binding. Supershift analysis with the highly specific antibody directed against C/EBPβ, C/EBPα, C/EBPδ, or AhR indicated that the C/EBP binding complex was comprised of C/EBPβ and partly C/EBPδ, but not AhR (Fig. 6B). Similar activation of C/EBP DNA binding was observed in cells treated with 10 μM oltipraz (data not shown). Activation of C/EBP was also confirmed by increases in band intensities of C/EBP protein complex with the consensus C/EBP binding oligonucleotide (Fig. 6C).

**Effect of C/EBP on 3-MC-inducible CYP1A1 Transactivation**—To correlate the activation of C/EBP with the inhibition of 3-MC induction of CYP1A1 by oltipraz, constitutively active C/EBP-specific dominant-negative mutant (AC/EBP) was expressed in cells treated with 3-MC in the presence or absence of oltipraz. Expression of AC/EBP significantly abolished the ability of oltipraz to suppress 3-MC induction of CYP1A1 (Fig. 6D).
Transfection with pCMV500, which was used as a control vector, allowed oltipraz to inhibit CYP1A1 induction by 3-MC (mock-transfection).

To further verify the role of C/EBP activation and suppression of AhR-mediated CYP1A1 induction, AC/EBP was expressed in combination with the pGL-CYP1A1-1195 luciferase reporter gene.
Inhibition of PAH Induction of CYP1A1 by Oltipraz

In this study, we found the intriguing inhibitory effect of oltipraz on CYP1A1 induction by PAH. In contrast to the AhR-mediated induction of CYP1A1 by 30 μM oltipraz, oltipraz at the concentration of 10 μM, at which AhR binding to the XRE was not detectable, inhibited 3-MC (30–50 nM)-inducible CYP1A1 expression. The band intensity of AhR binding to the XRE was attenuated by treatment of cells with oltipraz (10 μM), suggesting that oltipraz directly inhibits PAH-bound AhR activation of the XRE or interferes with AhR DNA binding through activation of other proteins (e.g., transcription factors or corepressors)(Fig. 8). AhR repressor (AhRR), a member of the superfamily bHLH/PAS transcription factors, has recently been identified as a negative factor that suppresses AhR-mediated gene expression (38). The AhRR was shown to compete with AhR for ARNT by forming AhRR/ARNT complex (39). Oltipraz may inhibit CYP1A1 gene transactivation by AhRR activation, which remains to be established.

Previous study from our laboratory showed that oltipraz induces nuclear translocation of C/EBPβ, but not C/EBPα, and stimulates C/EBPβ binding to the C/EBP-response element in the GSTA2 gene (11). In the present study, we demonstrated for the first time that C/EBPβ activation by a physiologically obtainable concentration of oltipraz (i.e., 10 μM) is associated with the negative regulation of PAH-inducible CYP1A1 gene transactivation (Fig. 8). This was further supported by suppression of CYP1A1 promoter-luciferase reporter gene induction. The negative role of C/EBP protein activation by its binding to the C/EBP binding site in the CYP1A1 promoter was further corroborated by reversal of oltipraz inhibition of CYP1A1 induction by AC/EBP transfection. AhR-mediated gene transactivation by physiologically obtainable PAH (i.e., 10–50 nM) is likely to be balanced with C/EBPβ-mediated negative regulation by oltipraz for CYP1A1 expression. Gel shift analysis in this study also provided evidence that oltipraz serves as a negative regulator of CYP1A1 expression involving PAH-bound AhR activation of the XRE. When we increased the
concentrations of 3-MC to 100 nM or above, the inhibitory effect of oltipraz for CYP1A1 induction by 3-MC was gradually abolished. The pathway of AhR activation and AhR-ARNT binding to the XRE by PAH (at the relatively high concentrations) for CYP1A1 induction was apparently very efficacious probably because the core binding motif of the XRE is present in multiple copies upstream of the CYP1A1 gene promoter (40), whereas a single C/EBP binding site is present within the −1.2-kb gene promoter as shown in the current study. The binding of ligand-activated AhR-ARNT heterodimer to the XRE seems to be more dominating at the high PAH concentrations than the antagonistic pathway involving C/EBPβ activation and its binding to the C/EBP binding site.

In the present study, oltipraz at 10 μM, which is a concentration effective for C/EBPβ activation, induced CYP1A1 with noticeable AhR binding to the XRE. Transfection of H4IIE cells with the plasmid encoding for C/EBPβ presumably homodimers binding may transactivate the gene via recruitment of coactivators (e.g. CBP/p300). It is likely that CYP1A1 induction by 10 μM oltipraz may have resulted from C/EBPβ-mediated transactivation of the CYP1A1 gene. The differential intrinsic activities between oltipraz and PAH for CYP1A1 induction at the physiologically relevant concentrations seem to be associated with the distinct signaling pathways involving C/EBPβ and AhR activation, respectively.

AhR-mediated CYP1A1 transcription was down-regulated by oxidative stress (e.g. hydrogen peroxide) via nuclear factor-1 site in the gene promoter (41). The C/EBP-mediated negative regulation by oltipraz of the AhR-induced CYP1A1 gene transcription in the present study constitutes a distinct pathway, which differs from oxidative stress-induced down-regulation of the CYP1A1 gene. In the study from our laboratory, we revealed that the signaling pathway of phosphoinositol-3-kinase regulated C/EBPβ-mediated GSTA2 induction by PD98059 or flavone (20). The potential cancer chemoprevention through dietary flavonoids may result from C/EBPβ-mediated transcriptional activation of the phase II enzyme. PD98059, a flavonoid compound, has been claimed to be a ligand for the AhR and to function as an AhR antagonist (42). The result of the present study is in line with the finding that PD98059 antagonizes AhR-mediated response (42).

In conclusion, we provide evidence that oltipraz suppresses 3-MC induction of the CYP1A1 gene expression and that activation of C/EBPβ by oltipraz contributes to suppression of PAH-inducible AhR-mediated CYP1A1 expression. We demonstrated that activation of C/EBPβ constitutes a distinct pathway for the XRE for the CYP1A1 gene transcription. The observation that oltipraz suppresses the potent and efficacious induction of CYP1A1 by 3-MC is at least in part consistent with its cancer chemopreventive effect. The current study brings additional insights into the chemopreventive effects of oltipraz to modify CYP1A1 gene regulation involved in procarcinogen metabolism.

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