INDUCTION OF CYTOCHROMES P450 1A1 AND 1B1 BY EMODIN IN HUMAN LUNG ADENOCARCINOMA CELL LINE CL5

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ABSTRACT:
Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) is an active compound of many laxative herbal drugs. The present study aimed to determine the effects of emodin on cytochrome P450 (P450)-dependent monooxygenases of human lung adenocarcinoma CL5 cells. Treatment of CL5 cells with 100 μM emodin for 24 h induced benzo[a]pyrene hydroxylation, 7-ethoxyresorufin O-deethylation, and 7-ethoxycoumarin O-deethylation activities of S9 fractions. Immunoblot analysis of CL5 S9 proteins revealed that emodin induced proteins immunorelated to P450s 1A1 and 1B1. Northern blot analysis of total cellular RNA showed that emodin induced P450s 1A1 and 1B1 mRNA levels in CL5 cells. These inductive effects on P450 monooxygenase activity, protein, and mRNA were concentration- and time-dependent. Addition of emodin to CL5 cell S9 inhibited its 7-ethoxycoumarin O-deethylation activity. Treatment of CL5 cells with 10 μM 3-methylcholanthrene for 24 h induced monooxygenase activity and P450s 1A1 and 1B1 proteins and mRNA levels. Treatment of the lung cells with 100 μM emodin or purpurin (1,2,4-trihydroxyanthraquinone) for 24 h produced greater induction of P450s 1A1 and 1B1 mRNA than did anthraflavic acid (2,6-dihydroxyanthraquinone) or anthraquinone. The emodin treatment induced P450s 1A1 and 1B1 mRNA in human lung carcinoma NCI-H322 and breast cancer MCF-7 cells. Emodin induced P450 1A1, but not 1B1, mRNA in human hepatoma HepG2 cells. The present study demonstrates that emodin is an inducer of P450s 1A1 and 1B1 protein and mRNA in human lung adenocarcinoma CL5 cells. Modulation of P450 by emodin may be an important factor affecting metabolism and toxicity of the hydroxyanthraquinone in humans.

Humans are exposed directly and indirectly to anthraquinones in medicinal and industrial applications (Sendelbach, 1989). Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) is an active compound of many laxative herbal drugs, such as aloe, senna, and rhubarb (Fig. 1). The hydroxyanthraquinone shows a variety of biological effects. For example, treatment with emodin, a protein tyrosine kinase inhibitor, suppressed the growth and transformation of human nonsmall-cell lung cancer cells and ras-transformed bronchial epithelial cells (Jayasuriya et al., 1992; Chan et al., 1993; Zhang et al., 1995). Emodin induced free radical production in human mononuclear cells and inhibited nuclear transcription factor-κB activation by tumor necrosis factor-α in human vascular endothelial cells (Huang et al., 1991; Kumar et al., 1998). These and other biological effects of emodin are regarded as the underlying causes for the antiviral, anticancer, and vasorelaxant activities of hydroxyanthraquinone.

The cytochrome P450 (P4501-dependent) monooxygenase system

1 Abbreviations used are: P450, cytochrome P450; PAHs, polycyclic aromatic hydrocarbons; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; mAb, monoclonal antibody; RT, reverse transcription; PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; 3-MC, 3-methylcholanthrene; Ah, aryl hydrocarbon.

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Fig. 1. Chemical structures of emodin and related compounds.

P450 is involved in oxidative metabolism to form 2-hydroxyemodin and ω-hydroxyemodin, as proposed by Tanaka et al. (1987) and Mueller et al. (1998).
aromatic hydrocarbons (PAHs), arylamines, and heterocyclic amines (Guengerich and Shimada, 1998). These carcinogen-metabolizing P450s are expressed in many tissues, including liver, kidney, and lung (Sutter et al., 1994). Biotransformation of emodin to the genotoxic intermediates 2-hydroxyemodin and 1α-hydroxyemodin requires P450 (Tanaka et al., 1987; Mueller et al., 1998) (Fig. 1). Treatment of rats with anthraquinone, 1-hydroxyanthraquinone, 1,4-dihydroxyanthraquinone, or anthraflavic acid (2,6-dihydroxyanthraquinone) induced P450 1A1 protein and monoxygenase activities in liver microsomes (Ayrton et al., 1988; Longo et al., 2000). Direct information regarding the ability of emodin, a trihydroxyanthraquinone derivative, to induce P450 1A1 or 1B1 remains unavailable.

The human lung cancer cell line provides an alternative system to study modulation of human pulmonary P450. Treatment of human lung carcinoma NCI-H322 and NCI-H358 cells with β-naphthoflavone or benzo[a]anthracene induced benzo[a]pyrene hydroxylation and 7-ethoxycozymar O-deethylation activities in cell lysate (Falzon et al., 1986). It is difficult to generalize the P450 induction properties of these lung cells because they show differential responses toward different P450 inducers.

Materials and Methods

Cell and Treatments. The human lung cancer cell line CL5 was derived from a lung adenocarcinoma tumor specimen of a 40-year-old woman patient at the Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. The cell line has been single-cell cloned and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 µM penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO₂. The cells were used when the monolayer had reached near confluence. Emodin was purchased from Sigma (St. Louis, MO) and dissolved in dimethyl sulfoxide (DMSO) and added to the medium so that DMSO concentration in the medium was less than 0.1%. Cell viability was determined using a trypan blue dye exclusion method. The cells were harvested by scraping and washed in a phosphate-buffered saline solution, pH 7.4. The following procedures were carried out at 4°C. Cell suspension was centrifuged at 300g for 3 min; the cell pellet was washed and homogenized in 0.1 M potassium phosphate buffer, pH 7.4. Cell homogenate was centrifuged at 9000g for 20 min, and the resulting supernatant, S9 fraction, was stored at −70°C before monoxygenase and immunoblot analyses.

Evaluation of Intracellular Peroxide Production. Peroxide production was determined using the oxidation-sensitive probe 2’,7’-dichlorofluorescein diacetate (DCFH-DA) (LeBel et al., 1992). Cells (1 x 10⁶/ml) were loaded with 5 µM DCFH-DA at 37°C before measurement and maintained in DCFH-DA continuously after. The level of intracellular peroxide was analyzed using a FACSCalibur system (BD Biosciences, San Jose, CA) with excitation and emission settings at 495 and 525 nm, respectively.

Enzyme Assays. 7-Ethoxycozymarin O-deethylation activity was determined by detecting the fluorescent product 7-hydroxycozymarin following the method of Greenlee and Poland (1978). Benzo[a]pyrene hydroxylation activity was determined by measuring the formation of phenolic metabolites according to the method of Nebert and Gelboin (1968). 7-Ethoxyresorufin O-deethylation activity was determined by measuring the formation of the fluorescent product resorufin, as described previously (Pohl and Fouts, 1980). Total cellular glutathione content was determined with the enzymatic-recycling assay based on glutathione reductase following the method of Tietze (1969).

Gel Electrophoresis and Immunoblotting. S9 proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane, as described elsewhere (Ueng et al., 2000). Immunodetection of P450 1A1 was carried out using a mouse monoclonal antibody (mAb) 1-12-3 raised against rat P450 1A1 (Park et al., 1986), which was kindly provided by Dr. Sang S. Park (Occupational Diseases Diagnosis and Research Center, Industrial Research Institute, Korea Industrial Safety Corporation, Inchon, Korea). Immunodetection of P450 1B1 was carried out using a rabbit polyclonal antibody prepared against a P450 1B1 peptide corresponding to a putative surface loop region epitope on the human P450 1B1 protein. The P450 1B1-specific blotting antibody kit and cell microsomes containing recombinant human P450 1B1 were purchased from GENTEST (Woburn, MA). Intensities of the immunoreactive bands were determined using an IS-1000 Digital Imaging System (Alpha Innotech Corporation, San Leandro, CA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR). For cDNA synthesis, 2 µg of total RNA was heated in a final volume of 10 µl with 2 µM random hexamer oligonucleotide at 70°C for 5 min, chilled on ice, and reverse transcribed in a final volume of 25 µl containing 1 mM each dNTP, 5 µl of 5X avian myeloblastosis virus buffer, 40 units of RNasin, and 10 units of avian myeloblastosis virus reverse transcriptase according to the manufacturer’s instructions (Promega, Madison, WI). Samples were incubated at 48°C for 1 h and subsequently denatured at 94°C for 2 min. PCR primers for P450s and β-actin were synthesized according to the published sequences (Table 1) by Invitrogen (Carlsbad, CA). PCR was carried out in a final volume of 25 µl containing 2 µl of 10-fold-diluted RT sample, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 µM each dNTP (Amresco, Solon, OH) in the presence of 200 nM each primer and 1 unit of Dynazyme (Finnzymes, Helsinki, Finland). Amplifications were performed using a DNA thermal cycler (PerkinElmer Instruments, Norwalk, CT) under the thermocycle conditions indicated in Table 1 with the following profiles: 2 min at 94°C before the first cycle, 45 s for denaturation at 94°C, 1 min for primer annealing.
EMODIN INDUCTION OF CYP1A1 AND CYP1B1 IN LUNG CELLS

Results

CL5 cells were treated with 100 μM emodin, 10 μM 3-MC, 50 μM β-naphthoflavone (β-NF), 1 mM phenobarbital (PB), 171 mM (1%) ethanol (ETOH), or 100 μM dexamethasone (DEX) for 24 h. Total RNA was isolated from the variously treated cells and subjected to RT-PCR analysis using primers specific for CYP1A1, CYP1B1, CYP2B6/7, and the internal standard control β-actin following the conditions described in Table 1. M represents DNA size markers; bp, base pair; C, control.

44x269]

1 min 30 s for primer extension at 72°C, and 7 min at 72°C after the last cycle. All reactions were conducted with β-actin primers as internal controls. PCR products were separated on 2% agarose gels and stained with ethidium bromide.

Total RNA isolation and RNA Blotting. Total RNA was isolated from CL5 cells following the method of Chomczynski and Sacchi (1987) and subjected to RNA-blotting procedures, as described previously (Ueng et al., 2000). In brief, a P450 1A1 cDNA probe was prepared from a human P450 1A1 3’-end cDNA clone (pPh-450-3’) obtained from American Type Culture Collection (Manassas, VA) (Jaiswal et al., 1985). A 360-base pair PCR product specific for P450 1B1 was prepared for detection of P450 1B1 mRNA, as described by Dohr et al. (1995). The P450s 1A1 and 1B1 probes were 32P-labeled using a commercial random primers DNA-labeling system (In-vitrogen). The RNA blot was hybridized to the 32P-labeled P450 cDNA probes and subjected to autoradiography procedures. The RNA blot was then de-probed and hybridized to a rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe, as an internal control for the amount of RNA. The intensities of RNA bands were quantitated with the aid of a digital imaging analysis system.

Statistical Analysis. The statistical significance of difference between control and treated cells was evaluated by Student’s t test. A p value <0.05 was considered statistically significant.

inducing agents and emodin caused no apparent or marginal effects on P450 2B6/7 mRNA level (bottom, lanes 1–7).

Treatment of CL5 cells with 5 to 20 μM emodin for 12 to 48 h showed no or minimal effects on cell viability as evaluated by MTT assay (Table 2). Treatment with 50 μM emodin for 24 h or 100 μM emodin for 24 and 48 h caused 19, 15, and 46% decreases of cell viability, respectively. Alternatively, these relative decreases in the amount of MTT product could reflect a cytostatic effect of emodin. Treatment with 1 to 100 μM emodin for 24 h induced 7-ethoxyco- marin O-deethylation activity of the S9 fraction in a concentration-dependent manner (Table 3). Treatment with 100 μM emodin for 24 h produced time-dependent induction of the monooxygenase activity. Based on these induction kinetic data, the following monooxygenase induction studies were carried out using CL5 cells treated with 100 μM emodin for 24 h. Additional cells were treated with 10 μM 3-MC for 24 h for comparison purposes. Emodin resulted in 7- and 2-fold increases of peroxide production and glutathione content in CL5 cells, respectively (Table 4). 3-MC had no effect on peroxide production but increased glutathione content by 2-fold. Emodin induced benzo[a]pyrene hydroxylation, 7-ethoxyresorufin O-deethyla- tion, and 7-ethoxyco- marin O-deethyla- tion in the S9 fraction. 3-MC also induced monooxygenase activities toward benzo[a]pyrene, 7-ethoxyresorufin, and 7-ethoxyco- marin. The induction of monooxygenase activities elicited by 3-MC was in general greater than the induction by emodin.

The effect of the addition of emodin to S9 on monooxygenase activity was determined using S9 fractions prepared from CL5 cells pretreated with 100 μM emodin or 10 μM 3-MC for 24 h. Additions of 1 to 100 μM emodin caused concentration-dependent inhibition of 7-ethoxyco- marin O-deethyla- tion activity of S9 fractions from emodin- or 3-MC-pretreated cells (Fig. 3). Concentrations of emodin causing 50% inhibition of 7-ethoxyco- marin O-deethyla- tion activity (IC50 in mean ± S.E.) of emodin- or 3-MC-pretreated cell S9 fractions were 23 ± 2 and 143 ± 23 μM, respectively.

CL5 cells were treated with emodin at increasing concentrations and times. S9 fractions and total RNA were prepared and subjected to immunoblot and RNA blot analysis, respectively. The results of immunoblot analysis showed that treatment with 50 or 100 μM emodin for 24 h induced the intensity of proteins immunorelated to P450s 1A1 and 1B1 (Fig. 4, lanes 5 and 6). Treatment with 1, 5, or 10 μM emodin did not show marked effects on P450s 1A1 and 1B1 proteins (lanes 2–4). Treatment with 100 μM emodin showed induction of P450s 1A1 and 1B1 proteins at 12 and 24 h (Fig. 5, lanes 5 and 6). The effects of emodin on P450s 1A1 and 1B1 proteins were not apparent at 6 h or earlier (lanes 2–4). The results of RNA blot analysis

![TABLE 2](http://dx.doi.org/10.1093/molc/1231.EMODIN+INDUCTION+OF+CYP1A1+AND+CYP1B1+IN+LUNG+CELLS)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>20</td>
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<td>54</td>
<td>54</td>
</tr>
<tr>
<td>50</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>100</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
</tbody>
</table>

* Value significantly different from the respective control value, p < 0.05.

CL5 cells were treated with emodin at the concentrations and times indicated. MTT assay was carried out using 96-well plates, as described under Materials and Methods. Viability was expressed as the ratio of absorbance at 570 nm of cells treated with emodin to that of control cells, which were treated with the vehicle DMSO only. Each value represents mean ± S.E. for five wells.

![Fig. 2](http://dx.doi.org/10.1093/molc/1231.EMODIN+INDUCTION+OF+CYP1A1+AND+CYP1B1+IN+LUNG+CELLS)

RT-PCR analysis of cytochromes P450 mRNA in human lung adenocarcinoma CL5 cells treated with emodin or P450 inducers.

CL5 cells were treated with 100 μM emodin, 10 μM 3-MC, 50 μM β-naphthoflavone (β-NF), 1 mM phenobarbital (PB), 171 mM (1%) ethanol (ETOH), or 100 μM dexamethasone (DEX) for 24 h. Total RNA preparations from control and the variously treated cells were subjected to RT-PCR analysis using the primers specific for CYP1A1, CYP1B1, CYP2B6/7, and the internal standard control β-actin following the conditions described in Table 1. M represents DNA size markers; bp, base pair; C, control.
Similar to emodin, treatment with 0.1 to 10 μM emodin, and S9 fractions were prepared at the times indicated. 7-Ethoxycoumarin O-deethylation activity was determined, as described under Materials and Methods. Each value represents mean ± S.E. for two experiments.

### TABLE 3

**Concentration- and time-dependent effects of emodin on 7-ethoxycoumarin O-deethylation activity in human lung adenocarcinoma CL5 cells**

<table>
<thead>
<tr>
<th>Emodin (μM)</th>
<th>7-Ethoxycoumarin O-deethylation (pmol of HC/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>50</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>100</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

* Value significantly different from the respective control value, p < 0.05.

**Effects of emodin and 3-methylcholanthrene on peroxide production, glutathione content, and drug-metabolizing enzyme activities in human lung adenocarcinoma CL5 cells**

CL5 cells were treated with 100 μM emodin or 10 μM 3-MC for 24 h. Control cells were treated with DMSO only. Cell homogenate and S9 fractions were prepared for total glutathione content and drug-metabolizing enzyme activity determinations, as described under Materials and Methods, respectively. In peroxide study, the cells were treated with 5 μM DCFH-DA for 2 h before analysis of fluorescence of DCFH using a flow cytometer. Each value represents mean ± S.E. for three experiments.

**TABLE 4**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Control</th>
<th>Emodin 3-MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxide production (fluorescence unit)</td>
<td>397 ± 16</td>
<td>2,174 ± 284*</td>
</tr>
<tr>
<td>Glutathione content (nmol/mg of protein)</td>
<td>19.6 ± 0.2</td>
<td>39.3 ± 2.8a</td>
</tr>
<tr>
<td>Benzopyrene hydroxylation (pmol of OHPB/min/mg of protein)</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.1*</td>
</tr>
<tr>
<td>7-Ethoxyresorufin O-deethylation (pmol of RF/min/mg of protein)</td>
<td>0.2 ± 0.1</td>
<td>2.5 ± 0.2*</td>
</tr>
<tr>
<td>7-Ethoxycoumarin O-deethylation (pmol of HC/min/mg of protein)</td>
<td>0.1 ± 0.0</td>
<td>1.6 ± 0.4*</td>
</tr>
</tbody>
</table>

* Value significantly different from the respective control value, p < 0.05.

showed that treatment with 50 or 100 μM emodin markedly induced the intensities of P450s 1A1 and 1B1 mRNA bands (Fig. 6 top and middle, lanes 5 and 6). Treatment with 1, 5, or 10 μM emodin caused no effect or minimal induction of P450s 1A1 and 1B1 mRNA (lanes 2–4). Treatment with 100 μM emodin induced P450s 1A1 and 1B1 mRNA levels at 3 h and thereafter (Fig. 7 top and middle, lanes 3–6). Similar to emodin, treatment with 0.1 to 10 μM 3-MC for 24 h or 10 μM 3-MC for 4 h showed concentration- and time-dependent induction of P450s 1A1 and 1B1 protein and mRNA, respectively (data not shown). Quantitative analysis of the intensities of the protein and mRNA bands showed that P450s 1A1 and 1B1 protein levels induced by treatment with 100 μM emodin for 24 h were about 3-fold less than the respective levels induced by 10 μM 3-MC (Table 5). Emodin and 3-MC induced P450 1A1 mRNA level by 16- and 17-fold and P450 1B1 mRNA by 8- and 7-fold, respectively.

CL5 cells were treated with 100 μM anthracene, anthrone, anthraquinone, anthraflavic acid, purpurin (1,2,4-trihydroxyanthraquinone), or emodin for 24 h. The results of RNA blot analysis showed that purpurin markedly induced the intensity of P450s 1A1 and 1B1 mRNA bands, similar to emodin (Fig. 8 top, lanes 5 and 6). Anthraflavic acid caused minimal induction of P450s 1A1 and 1B1 mRNA levels (lane 7). Anthracene, anthrone, and anthraquinone had no or minimal effects (lanes 2–4). NCI-H322, human breast MCF-7, and hepatoma HepG2 cells were treated with 100 μM emodin for 24 h, total RNA was prepared and subjected to RNA blot analysis. The results showed that emodin produced 8-, 7-, and 4-fold induction of P450 1A1 mRNA and 6-, 3-, and 3-fold induction of P450 1B1 mRNA levels in CL5, NCI-H322, and MCF-7 cells, respectively (Table 6). Emodin induced P450 1A1, but not P450 1B1, mRNA in HepG2 cells. Treatment of these cell lines with 10 μM 3-MC for 24 h

**FIG. 3.** Effects of the addition of emodin on 7-ethoxycoumarin O-deethylation activity of S9 fractions from human lung adenocarcinoma CL5 cells pretreated with emodin or 3-methylcholanthrene.

S9 fractions were prepared from CL5 cells treated with 100 μM emodin or 10 μM 3-MC (●) for 24 h and used for determinations of 7-ethoxycoumarin O-deethylation activity, as described under Materials and Methods. Emodin was added to the monoxygenase assay system at the concentrations indicated. Each data point represents mean ± S.E. for three experiments.

**FIG. 4.** Concentration-response relationships of effects of emodin on cytochromes P450 1A1 and 1B1 proteins in human lung adenocarcinoma CL5 cells.

CL5 cells were treated with emodin at the concentrations indicated for 24 h. S9 proteins from the treated cells were subjected to protein blot analysis in which a mAb 1-12-3 to rat and human P450 1A1 and a rabbit polyclonal antibody against human P450 1B1 were used to probe for immunorelated proteins, as described under Materials and Methods. The protein load in each lane was 100 μg.

**FIG. 5.** Time courses of effects of emodin on cytochromes P450 1A1 and 1B1 proteins in human lung adenocarcinoma CL5 cells.

CL5 cells were treated with 100 μM emodin for the times indicated. S9 proteins from the treated cells were subjected to protein blot analysis in which a mAb 1-12-3 to rat and human P450 1A1 and rabbit polyclonal antibody against human P450 1B1 were used to probe for immunorelated proteins as described under Materials and Methods. The protein load in each lane was 100 μg.
CL5 cells were treated with emodin at the concentrations indicated for 24 h. Total RNA from the treated cells was subjected to RNA blot analysis in which a $^{32}$P-labeled P450 1A1 or 1B1 cDNA probe was used to detect hybridizable mRNA species, as described under Materials and Methods (top and middle). RNA blot was reprobed using a $^{32}$P-labeled GAPDH cDNA probe as an internal control. A representative GAPDH blot is shown in the figure (bottom). The RNA load in each lane was 20 µg.

![Image](88x381 to 252x501)

**Fig. 6.** Concentration-response relationships of effects of emodin on cytochromes P450 1A1 and 1B1 mRNA levels in human lung adenocarcinoma CL5 cells.

CL5 cells were treated with 100 µM emodin for the times indicated. Total RNA was isolated and subjected to RNA blot analyses in which a $^{32}$P-labeled P450 1A1 or 1B1 cDNA probe was used to detect hybridizable mRNA species, as described under Materials and Methods (top and middle). RNA blot was reprobed using a $^{32}$P-labeled GAPDH cDNA probe as an internal control. A representative GAPDH blot is shown in the figure (bottom). The RNA load in each lane was 20 µg.

![Image](91x611 to 250x729)

**Fig. 7.** Time-courses of effects of emodin on cytochromes P450 1A1 and 1B1 mRNA levels in lung adenocarcinoma CL5 cells.

The preferential induction of P450 1B1 mRNA by emodin in CL5 and other extrahepatic cells, but not in HepG2, indicates complexity in regulation of P450 1B1 gene expression in these cell lines. Kress and Greenlee (1997) reported a cell-specific regulation of P450s 1A1 and 1B1 in HepG2 and human renal adenocarcinoma ACHN cell lines in which 2,3,7,8-tetrachlorodibenzo-p-dioxin preferentially induced P450 1A1 in HepG2 cells and P450 1B1 in ACHN cells. They suggested that a repression of 2,3,7,8-tetrachlorodibenzo-p-dioxin-dependent P450 1A1 induction in ACHN cells occurred at the level of transactivation in the Ah receptor signal transduction pathway because of the data from gel shift analysis of P450 1A1 dioxin-responsive element binding and intact cell dioxin-responsive element footprinting. It will be of interest to investigate whether an analogous repressive mechanism occurs with P450 1B1 in HepG2 cells treated with emodin.

**Discussion**

Pulmonary xenobiotic-metabolizing enzymes are active predominantly in Clara cells and less in alveolar type II cells. The ciliated bronchial epithelial cells, alveolar macrophages, and capillary endothelial cells are the other metabolically active populations (Foth, 1995). The present study has partially characterized human xenobiotic-metabolizing enzymes in CL5 derived from a bronchial epithelium cell type. The presence of P450 enzymes in CL5 cells may have toxicological implications because bronchial epithelial cells are the progenitors of bronchogenic carcinomas, and induction of the carcinogen-metabolizing P450s may play a role as a metabolic determinant of cancer susceptibility of these lung cells. This possibility remains to be tested using normal cells in the trachea or the airways treated with pulmonary carcinogens.

The present finding demonstrates that emodin has the ability to induce P450s 1A1 and 1B1 in human lung adenocarcinoma cells. This is supported by the evidence that emodin induces P450s 1A1 and 1B1 protein and mRNA levels. Emodin also induces monooxygenase activities toward benzo[a]pyrene and 7-ethoxyresorufin, which are substrates of P450s 1A1 and 1B1 (Ryan and Levin, 1990; Shimada et al., 1997). Emodin induction of P450s 1A1 and 1B1 may occur via a mechanism involving binding of the inducer to an aryl hydrocarbon (Ah) receptor, a transcriptional factor of P4501A1 and P4501B1 genes (Whitlock, 1999). Our data show that treatment with emodin or purpurin resulted in marked induction of P450s 1A1 and 1B1 mRNA; in contrast, anthraflavic acid did not (Fig. 8). Additional study of the structure-activity relationship is required to determine the roles of the number and regiospecificity of the hydroxyl groups on the anthraquinone moiety in the modulation of binding of hydroxyanthraquinones to the Ah receptor and possibly biological responses of the receptor. The present data do not exclude the possibility that emodin may induce P450 by mechanisms that do not involve binding of the inducer to the Ah receptor. For example, emodin may activate the Ah receptor complex via intracellular signal transduction systems in CL5 cells, like the mechanism of omeprazole induction of P450 1A1 in rat hepatoma H4IE cells (Backlund et al., 1997).

P450s 1A1 and 1B1 mRNA levels induced by 100 µM emodin or 10 µM 3-MC were similar; however, the P450 protein levels induced by emodin were markedly lower than the levels induced by 3-MC (Table 5). These inconsistent mRNA and protein effects suggest several interesting possibilities compatible with the regulatory mechanisms reported in the literature. First, emodin or its metabolite(s) are more capable of destroying P450 proteins relative to 3-MC, Souček (1999) showed that incubation of quinones with rat liver microsomes led to the destruction of P450s 1A and 3A protein. Second, emodin produced a greater oxidative stress than did 3-MC (Table 4), which resulted in a greater down-regulation of P450 protein expression. Previous studies reported that rhein, a dihydroxyanthraquinone derivative, and stress chemicals inhibited incorporation of amino acids to protein in mouse neoplastic cells and human cervical carcinoma HeLa cells, respectively (Duncan and Hershey, 1987; Castiglione et al., 1990). Third, emodin caused a greater degree of phosphorylation and consequently degradation of P450 protein than did 3-MC (Table 4), which resulted in a greater down-regulation of P450 protein expression. Previous studies demonstrated that glucagon or 8-bromoadenosine 3',5'-cyclic monophosphate enhanced the rate of phosphorylation of P450 and the consequent denaturation and degradation of the protein in vitro.

The preferential induction of P450 1B1 mRNA by emodin in CL5 and other extrahepatic cells, but not in HepG2, indicates complexity in regulation of P450 1B1 gene expression in these cell lines. Kress and Greenlee (1997) reported a cell-specific regulation of P450s 1A1 and 1B1 in HepG2 and human renal adenocarcinoma ACHN cell lines in which 2,3,7,8-tetrachlorodibenzo-p-dioxin preferentially induced P450 1A1 in HepG2 cells and P450 1B1 in ACHN cells. They suggested that a repression of 2,3,7,8-tetrachlorodibenzo-p-dioxin-dependent P450 1A1 induction in ACHN cells occurred at the level of transactivation in the Ah receptor signal transduction pathway because of the data from gel shift analysis of P450 1A1 dioxin-responsive element binding and intact cell dioxin-responsive element footprinting. It will be of interest to investigate whether an analogous repressive mechanism occurs with P450 1B1 in HepG2 cells treated with emodin.

The present study showed that addition of emodin inhibited 7-ethoxycoemurin O-deethylase activity of CL5 S9 fractions (Fig. 3), similar to previous studies showing that additions of emodin, anthraflavic acid, and purpurin inhibited P450 1A1-dependent monooxygenase activities of rat liver microsomes (Hao et al., 1995; Marczylo et al., 2000). These effects in vitro suggest a direct hydroxyanthraquinone and P450 interaction producing the inhibition of monooxygenase activity. Addition of purpurin to rat liver
and RNA-blotting analyses were carried out, as described under Materials and Methods. Intensities of protein and mRNA bands were quantitated using a digital image analyzer. Intensity of the P450 mRNA band was normalized against internal standard GAPDH. Each value represents mean ± S.E. for three experiments.

Comparison of effects of emodin and 3-methylcholanthrene on cytochromes P450 1A1 and 1B1 protein and mRNA levels in human lung adenocarcinoma CL5 cells

CL5 cells were treated with 100 μM emodin or 10 μM 3-MC for 24 and 6 h for S9 protein and total RNA preparations, respectively. Control cells were treated with DMSO only. Protein and RNA-blotting analyses were carried out, as described under Materials and Methods. Intensities of protein and mRNA bands were quantitated using a digital image analyzer. Intensity of the P450 mRNA band was normalized against internal standard GAPDH. Each value represents mean ± S.E. for three experiments.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Emodin</th>
<th>3-MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>N.D.</td>
<td>22 ± 1*</td>
<td>75 ± 3*</td>
</tr>
<tr>
<td>mRNA</td>
<td>0.23 ± 0.01</td>
<td>3.68 ± 0.65*</td>
<td>3.83 ± 0.50*</td>
</tr>
</tbody>
</table>

N.D., not detectable.

* Value significantly different from the respective control value, p < 0.05.

![Effect of Emodin and 3-Methylcholanthrene on Cytochromes P450 1A1 and 1B1 mRNA Levels in Human Lung Adenocarcinoma CL5 Cells](image)

In conclusion, the present study demonstrates that emodin is an inducer of P450s 1A1 and 1B1 in human lung adenocarcinoma CL5 cells. The ability of emodin to induce and inhibit P450-dependent catalytic activity may be an important factor to consider in the assessment of drug interaction and toxicity susceptibility associated with human exposure to the hydroxyanthraquinone.

Acknowledgments. We thank Dr. Sang S. Park for the monoclonal antibody P450 1A1.

Effects of emodin and 3-methylcholanthrene on cytochromes P450 1A1 and 1B1 mRNA levels in human tumor derived cells

Human lung adenocarcinoma CL5, lung carcinoma NCI-H322, breast cancer MCF-7, and hepatoma HepG2 cells were treated with 100 μM emodin or 10 μM 3-MC for 24 h. Total RNA was prepared and subjected to RNA blot analyses using probes specific for P450 1A1 and 1B1, as described under Materials and Methods. Intensity of the P450 mRNA band was normalized against internal standard GAPDH. Each value represents mean ± S.E. for at least four experiments.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Emodin</th>
<th>3-MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL5</td>
<td>0.40 ± 0.10</td>
<td>3.14 ± 0.50*</td>
<td>3.32 ± 0.42*</td>
</tr>
<tr>
<td>NCI-H322</td>
<td>0.13 ± 0.03</td>
<td>0.94 ± 0.12*</td>
<td>1.10 ± 0.20*</td>
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<tr>
<td>MCF-7</td>
<td>0.44 ± 0.06</td>
<td>1.63 ± 0.20</td>
<td>2.15 ± 0.20*</td>
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<tr>
<td>HepG2</td>
<td>0.20 ± 0.07</td>
<td>1.79 ± 0.31*</td>
<td>1.81 ± 0.18*</td>
</tr>
</tbody>
</table>

N.D., not detectable.

* Value significantly different from the control value, p < 0.05.


References


