Post-Translational Inhibition of Cytochrome P-450 2E1 Expression by Chlomethiazole in Fao Hepatoma Cells

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ABSTRACT

Chlomethiazole (CMZ) is a sedative and anticonvulsant drug of expression of rat hepatic ethanol-inducible cytochrome P-450 2E1 (CYP2E1). Recent results have shown that human CYP2E1 expression in vivo is almost completely inhibited in control subjects and in alcoholic patients treated with CMZ. In the present investigation, we evaluated the mode of action of CMZ on CYP2E1 expression in Fao rat hepatoma cells. Transcriptional activity of the CYP2E1 gene was monitored using reverse transcription-polymerase chain reaction-based quantification of CYP2E1 heterologous nuclear RNA (hnRNA) against a mimic DNA standard, mRNA was detected by Northern blotting, enzyme protein was detected by Western blotting, and CYP2E1-dependent catalytic activity was detected by assay of chlorzoxazone-6-hydroxylation. Six hours after CMZ treatment, the levels of both CYP2E1 protein and catalytic activity were concomitantly reduced at an IC50 value of about 5 μM. Ethanol treatment of the cells caused a 2-fold induction of CYP2E1 protein levels, which was inhibited by CMZ. Change of medium unexpectedly caused an increase in CYP2E1 gene transcription 4 h later, as monitored by quantitative determination of CYP2E1 hnRNA. However, CMZ failed to influence the expression of CYP2E1 hnRNA or mRNA both constitutively and after medium change, indicating no effect on gene transcription or mRNA synthesis/stability. Cycloheximide treatment of the cells did not abolish the inhibitory action of CMZ, further indicating an action at the post-translational level; in addition, CMZ inhibited CYP2E1 expression in V79 cells with stably expressed CYP2E1 under the control of the SV40 promoter. The data indicate that the CYP2E1 gene is transcriptionally activated in response to medium change and that CMZ, apart from a transcriptional inhibitor of CYP2E1 expression, acts in addition as an efficient high-affinity post-translational inhibitor of CYP2E1, probably due to an allosteric destabilization of the enzyme. This indicates a very rapid and effective CMZ-mediated inhibition of CYP2E1 in vivo.

Ethanol-inducible cytochrome P-450 2E1 (CYP2E1) is constitutively expressed in liver and many other organs. The enzyme is effectively induced by a diverse set of chemicals, including ethanol, isoniazid, and acetone. CYP2E1 metabolizes several different classes of compounds, in particular, small and hydrophobic substances. Among the CYP2E1 substrates are organic solvents, acetaminophen, volatile anesthetics, dimethylsulfoxamine, ethanol, and acetaldehyde (Ronis et al., 1991). A unique property of CYP2E1 is its ability to produce reactive oxygen radicals, which can initiate microsomal lipid peroxidation (Ekstro¨m and Ingelman-Sundberg, 1989), a process that appears to be important in the etiology of alcoholic liver disease (Castillo et al., 1992; Ingelman-Sundberg et al., 1993; Morimoto et al., 1993; Morimoto et al., 1995; French et al., 1998). Oxidative stress caused by the expression of the enzyme in, for example, HepG2 cells, which normally lack this enzyme, leads to cell death (Chen and Cederbaum, 1998).

CYP2E1 can be regulated at different cellular levels. The gene is transcriptionally activated after birth and during starvation (Song et al., 1986; Johansson et al., 1990; Ueno and Gonzalez, 1990; Hu et al., 1995); the mRNA appears to be stabilized under diabetic conditions (Song et al., 1987), whereas the major level of regulation is post-translational, by which all substrates and ligands protect the enzyme from degradation (Eliasson et al., 1990; Ueshima et al., 1993; Eliasson and Kenna, 1996). A number of mechanism-based inhibitors of CYP2E1 have been described in the literature, including allylmercaptan (Kwak et al., 1994), 3-amino-1,2,4-triazole (Koop, 1990), di-allylsulfide (Brady et al., 1991a), disulfiram (Brady et al., 1991c,b), and phenethylisothiocyanate (Ishizaki et al., 1990). These inhibitors are not specific for CYP2E1 and thus are less useful for selective inhibition in vivo. Chlomethiazole (CMZ) has, however, been shown to be quite selective for CYP2E1 inhibition. This compound, which possesses seda-
YH439, a thiazole-related compound, has been shown to be induced by starvation in rats (Hu et al., 1994). In addition, YH439, a thiazole-related compound, has been shown to effectively inhibit CYP2E1 expression in vivo in rats (Jeong et al., 1995).

In humans, CYP2E1 participates in the metabolism of drugs like chlorozoxazine (CZN), acetaminophen, and volatile anesthetic agents. The activity of CYP2E1 in vivo can be monitored by measuring the ratio between 6-hydroxy-CZN and the parent compound in blood (Girre et al., 1994). It can be argued that inhibition of CYP2E1 in vivo might be favorable to reduce the formation of several hepatotoxic compounds caused by CYP2E1-dependent bioactivation, as well as to diminish the extent of oxidative stress as a result of alcohol consumption, thereby reducing the hepatotoxic effects of ethanol. Indeed, CMZ has recently been shown to almost completely ameliorate liver damage in rats treated chronically with ethanol in the total enteral nutrition model (French et al., 1998).

Recently, Gebhardt et al. (1997) showed that CMZ effectively inhibited CYP2E1 expression in vivo in humans as monitored by CZN-6-hydroxylation, suggesting the potential of this compound as an efficient tool for this purpose. In contrast to our results obtained in rat liver microsomes (Hu et al., 1994; Gebhardt et al. 1997) found that CMZ acts as a noncompetitive inhibitor of CYP2E1 activity with a Ki value of 12 μM. To investigate the basis for further mechanisms of inhibition of CYP2E1 by CMZ, we used the Fao hepatoma cell line, which constitutively expresses CYP2E1 (de Waziers et al., 1995). The data are consistent with a mechanism of inhibitory action of CMZ entirely at the post-translational level, which constitutes a novel mechanism of action for this compound.

**Experimental Procedures**

**Materials.** CMZ was a gift from Astra Arcus AB (Södertälje, Sweden). DMEM, FBS, and penicillin/streptomycin were purchased from Life Technologies Europe (Paisley, Scotland, UK). F12 Coon’s modification medium and other chemicals were purchased from Sigma Chemical (Poole, Dorset, UK). Antisera against CYP2E1 and NADPH:cytochrome P-450 reductase were accomplished through immunization in rabbits as described previously (Johansson et al., 1988; Johansson and Ingelman-Sundberg, 1988; Neve et al., 1996). Protein A-conjugated horseradish peroxidase and bisacylamide solution were from Bio-Rad (Hercules, CA). Hybond-C nitrocellulose membranes and an enhanced chemiluminescence kit were purchased from Amersham (Buckinghamshire, UK).

**Cell Culture.** Fao cells derived from Reuber H35 rat hepatoma (de Waziers et al., 1992) were maintained in F12 Coon’s modification medium supplemented with 5% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were grown on 60-mm petri dishes at 37°C, and the medium was changed every 2 to 3 days and always 1 h before stimulation. In the ethanol experiments, the media were changed every 12 h within the treatment period. In cycloheximide (CHX) experiments, the medium was changed 1 h before CHX administration and CMZ was administered simultaneously. As reported previously by Zhukov and Ingelman-Sundberg (1997), used in pulse-chase experiments, a concentration of CHX equal to 10 μg/ml caused a greater than 95% inhibition of protein synthesis.

V79 hamster fibroblasts, stably transfected with rat CYP2E1 cDNA (Schmalix et al., 1995), were maintained in DMEM containing 1000 mg/liter glucose, 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured in 60-mm petri dishes at 37°C, and the medium was changed every 3 to 4 days and always 24 h before stimulation.

**Isolation of Microsomal Fraction.** The cell medium was discarded, and the cells were washed twice with 8 mM Na2HPO4, 1.5 mM KH2PO4, 2.7 mM KCl, and 140 mM NaCl and harvested in the same buffer. At this point, the cells could be stored at −70°C for several days. After thawing, the cells were pelleted at 4500g for 3 min at 4°C. The cell pellet was resuspended in 100 mM potassium phosphate and 10 mM EDTA, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride, and the cells were sonicated for 20 × 3 s. The cell lysate was pelleted at 10,700g for 10 min at 4°C. The cells were resuspended in the same buffer, sonicated for 6 × 2 s, and pelleted at 10,000g for 10 min at 4°C. The two supernatants were pooled and centrifuged at 85,000g for 60 min at 4°C. The microsomal pellet was suspended in 50 mM Na2HPO4, pH 7.4, containing 0.1 mM EDTA and 10% glycerol.

**Immunoassay.** The samples were subjected to SDS-polyacrylamide gel electrophoresis in a Bio-Rad Mini-Protein gel (10% bisacrylamide, 15 μg protein/well) and transferred onto a nitrocellulose membrane in the Towbin buffer system (Towbin et al., 1979). After completion of transfer, the membrane was dried; re-soaked in 50 mM Tris, pH 7.5, 0.2 M NaCl, and 0.05% Tween 20; blocked in the same buffer containing 5% nonfat dry milk for 1 h; incubated with the antisera for 1 h; and then incubated for 1 h with protein A-conjugated horseradish peroxidase, both diluted 1:1000 in these buffer containing 1% milk. The bands were visualized using an enhanced chemiluminescence kit (Amersham) under conditions that showed linearity with respect to the amount of protein. The membrane was reprobed with an antisera against P-450-reductase (diluted 1:2000), and the CYP2E1 content was expressed in relation to the reductase signal as a standard as described by Zhukov and Ingelman-Sundberg (1997).

**Determination of CYP2E1 Activity by CZN-6-Hydroxylation Assay.** CZN-6-hydroxylation experiments were carried out as described previously (Tindberg and Ingelman-Sundberg, 1996). Briefly, 500 μM CZN was incubated with 200 μg of microsomal protein in the presence of 0.5 mM NADPH in a 50 mM NaHPO4 buffer, pH 7.4, containing 0.1 mM EDTA. After a 45-min incubation at 37°C, the reaction was quenched by the addition of 50 μl of 43% phosphoric acid, and the internal standard was added (50 ng of acetaminophen). Incubations of a total volume of 1 ml were extracted twice with 2 and 1 ml of dichloromethane, and the phases were separated after a 10-min centrifugation at 3000g. The combined organic phases were dried under nitrogen flow and dissolved in 50 μl of mobile phase (acetoni-trile/0.5% phosphoric acid, 22:78), and 15-μl aliquots were used for HPLC analysis. The CYP2E1 activity (generation of 6-OH CZN) was calculated as percentage of the control activity in cells without treatment.

**RNA Preparation and Northern Blot Analysis.** Total RNA was isolated from cells cultured on 100-mm petri dishes according to the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987). The RNA was subjected to electrophoresis on a 1.2% agarose/formaldehyde denaturing gel (25 μg/lane), transferred onto a nitrocellulose filter, and hybridized with radiolabeled cDNA for CYP2E1 and β-actin, as described previously (Struhl, 1990). The probes were obtained by RT-PCR amplification of rat liver RNA and were labeled with α-32P-dCTP (3000 Ci/mmol; Amersham) using the Megaprime DNA labeling system (Amersham).

**Intron-Specific Reverse Transcription-Polymerase Chain Reaction Assay.** Total RNA (1 μg) was reverse transcribed using an intron-specific reverse transcription (RT) primer corresponding to the intron 2, 3258- to 3277-bp sequence 5′-CAAC TG7 GTC GGC CTG AATC-3′; of the rat CYP2E1 gene, together with an oligo(dT) primer. RT reaction was performed at 50°C, using the 1st Strand
cDNA synthesis kit (Clontech, Palo Alto, CA). Quantitative polymerase chain reaction (PCR) analysis of the relative amount of the CYP2E1 heterologous nuclear RNA (hnRNA) in differently treated Fao cells was performed using the PCR MIMIC 228 kit (Clontech). The primers used in the PCR analysis corresponded to the intron 1, 2415- to 2434-bp sequence 5' -GTG AAG TAC AGT ACA GGA GC-3' for the upstream primer, and the intron 2, 2954- to 2973-bp DNA sequence 5' -CT CGT GCT TTC CTA CAG TTC-3' for the downstream primer. The CYP2E1 hnRNA fragment, which is 558 bp long, was amplified in the presence of decreasing concentrations of a 320-bp DNA standard (MIMIC DNA, constructed by PCR amplification of a BamH1/EcoRI restriction fragment of the v-erbB gene, as described in the PCR MIMIC construction kit). The PCR amplification was conducted in a Perkin-Elmer amplifier, under the following thermal cycle conditions: 45 s denaturing at 94°C, 45 s annealing at 62°C, and 1 min extension at 72°C, for 37 cycles, followed by 7 min final extension at 72°C. The PCR products were analyzed on a 1.6% agarose/TBE gel, and the negatives of Polaroid photographs of the gels stained with ethidium bromide were scanned using a laser densitometer. The levels of CYP2E1 hnRNA were calculated as the concentration of the mimic DNA required to reach an equal density of the target and mimic bands by examining the relative intensities between target and mimic in reactions carried out at three or four different mimic DNA concentrations. The results were normalized by the β-actin content of each sample, which was also quantified by the RT-PCR MIMIC system (for primer sequence and experimental setup, see Fang et al., 1998).

Results

Characterization of CYP2E1 Apoprotein in Microsomal Fractions of Fao Cells Treated with CMZ. Fao hepatoma cells were treated with CMZ, and the CYP2E1 expression was monitored in isolated microsomes by Western blotting and analysis of the rate of 6-hydroxylation of the CYP2E1 substrate CZN. CMZ administered at a dose of 100 μM did not influence the CYP2E1 expression until 6 h after the treatment, when a pronounced decline in both protein (Fig. 1) and catalytic activity (Table 1) was registered. A single addition of the drug to the cells was sufficient to suppress CYP2E1 protein levels for more than 24 h (Fig. 1); indeed, the protein levels of CYP2E1 did not recover even after 72 h of treatment (not shown). These results were in good agreement with the levels of CYP2E1 activity, as monitored by the 6-hydroxylation of CZN in Fao cell microsomes (Table 1). This indicates a primary action of CMZ at the level of the enzyme itself. By contrast, 100 μM CMZ did not influence the expression of either NADPH:cytochrome P-450 reductase (Fig. 1) or CYP2B1 (data not shown). No cytotoxicity was detected even when a dose of 300 μM CMZ was used.

Effect of CMZ on CYP2E1 mRNA and hnRNA in Fao Cells. Previous results have shown that determination of P-450 hnRNA expression constitutes a reliable method for the measurement of the gene transcriptional activity (Correllas and Reiners, 1996; Backlund et al., 1997). The effect of CMZ on CYP2E1 expression at the transcriptional level was determined by quantitative RT-PCR-based determination of CYP2E1 hnRNA using a 558-bp fragment corresponding to exon 2 and parts of the flanking intron sequences of the CYP2E1 gene as a target, coamplified with a part of the v-erbB gene as a MIMIC DNA standard. CYP2E1 mRNA was quantified using Northern blotting analysis and a CYP2E1-specific cDNA probe (see Experimental Procedures). In both cases, β-actin expression was used as an internal standard. The assay of hnRNA expression revealed an expected proportional competition between the target and mimic (Fig. 2), the absence of contamination, and the expected size of the products using target only, mimic only, and genomic DNA as templates. As shown in Fig. 2, a change in cell medium caused an elevated expression of CYP2E1 hnRNA, which was detected even when a dose of 300 μM CMZ was used.

![Fig. 1. Time and dose dependence of constitutive CYP2E1 expression on CMZ treatment in Fao cells. A, immunoblot analysis was performed in microsomes isolated from Fao cells treated with and without CMZ (at a dose of 100 μM), using anti-CYP2E1 and anti-reductase sera. Each point on the diagram is the mean ± S.E.M. of at least four independent experiments and represents the percentage of the control value that remains after treatment with CMZ for the indicated time periods. The values were obtained by densitometric scanning and ImageQuant software program and are divided by the scanning values of NADPH:cytochrome P-450 reductase. B, CMZ dose-dependent reduction of CYP2E1 apoprotein in Fao cells treated with CMZ for 6 h. The IC50 value (or half-maximal dose) was calculated to be at 5.3 μM. C, representative immunoblot from Fao cells treated with or without CMZ for the indicated time points. Cell medium was changed 1 h before treatment. Total microsomal protein (15 μg/well) was subjected to SDS-polyacrylamide gel electrophoresis and hybridized after transfer to nitrocellulose filter with anti-CYP2E1 (bottom) or anti-reductase (top) sera. Lanes 1 and 2, control; lanes 3 and 4, CMZ for 2 h; lanes 5 and 6, control; lanes 7 and 8, CMZ for 4 h; lanes 9 and 10, control; and lanes 11 and 12, CMZ for 8 h.](Image 308x542 to 559x729)

![Table 1](https://example.com/table1.png)

<table>
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<tr>
<th>Time</th>
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<th>% of 2-h Control</th>
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<td>98 ± 8</td>
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<td>4</td>
<td>Control</td>
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<td></td>
<td>CMZ</td>
<td>133 ± 12</td>
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<td>Control</td>
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<td>Control</td>
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<td>CMZ</td>
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*100% value corresponds to the rate of 6-OH-CZN formation equal to 27.5 pmol/mg protein/min.
quired de novo protein synthesis, we conducted experiments in which the cells were treated with the protein synthesis inhibitor CHX, together with CMZ. CHX alone, at a dose of 10 \( \mu \)g/ml, decreased the CYP2E1 protein levels to approximately 50% of the control after 6 h, as monitored by Western blotting. CMZ caused, however, a further decrease in the CYP2E1 protein levels in the presence of CHX, indicating an effect of the drug on the degradation rate of the protein. In both the absence and the presence of CHX, the protein levels were decreased by 50 to 60% at 6 h after initiation of the CMZ treatment (Fig. 3). The expression of NADPH:cytochrome P-450 reductase was not affected by this short CHX treatment, as expected from its longer half-life, and was used as an internal standard (Fig. 3). These results thus strongly indicate the mechanism of CMZ inhibition was exerted primarily at the post-translational level.

**Effect of CMZ on CYP2E1 Apoprotein in Stably Transfected V79 Cells.** To further evaluate the post-translational action of CMZ on CYP2E1 expression, we used stably transfected V79 cells expressing CYP2E1 under the control of the SV40 promoter (Schmalix et al., 1995). CMZ administered at a dose of 63 \( \mu \)M potently inhibited the constitutive CYP2E1 expression in these cells. The cellular levels decreased by 50% to 60% at 6 h after initiation of the CMZ treatment (Fig. 3). The expression of NADPH:cytochrome P-450 reductase was not affected by this short CHX treatment, as expected from its longer half-life, and was used as an internal standard (Fig. 3). These results thus strongly indicate the mechanism of CMZ inhibition was exerted primarily at the post-translational level.

**Fig. 2.** Effect of CMZ on CYP2E1 mRNA and \( hhn \)RNA levels in Fao cells. A, total RNA was isolated from Fao cells treated with or without CMZ for the indicated time periods. The RNA (25 \( \mu \)g/well) was subjected to formaldehyde denaturing gel electrophoresis and Northern blot analysis using \( ^{32} \)P-labeled probes for CYP2E1 (top) and \( \beta \)-actin (bottom). Lanes 1 and 2, control; lanes 3 and 4, CMZ for 2 h; lanes 5 and 6, control; lanes 7 and 8, CMZ for 4 h; lanes 9 and 10, control; and lanes 11 and 12, CMZ for 8 h. B, total RNA was reverse transcribed using both oligo(dT) and the intron-specific CYP2E1 RT primer and amplified in the presence of different concentrations of a standard DNA competitor (MIMIC DNA) for 37 cycles. The PCR products were analyzed on a 1.6% agarose/TBE gel and visualized with UV light using EtBr staining. Lane 1, molecular weight markers VIII; lanes 2 to 4, control sample amplified in the presence of decreasing concentrations of MIMIC DNA (3-fold dilutions); lanes 5 to 7, CMZ-treated sample amplified in the presence of decreasing concentrations of MIMIC DNA (the same 3-fold dilution as in the control sample); lane 8, negative control without any DNA template; lane 9, control with only target DNA as template; lane 10, control with only MIMIC DNA as template; negative control using as template nontranscribed total RNA from Fao cells; and lane 11, positive control using as template 100 ng of genomic DNA isolated from rat liver. C, values of the diagram represent the relative amount of CYP2E1 mRNA and \( hhn \)RNA in Fao cells treated with and without CMZ for the indicated time points. The data were derived from densitometric scanning of Northern blots or Polaroid picture negatives of EtBr-agarose gels, divided in both cases by the \( \beta \)-actin content of each sample and normalized to the control sample at 2 h of treatment. The values are mean ± S.E.M. of three independent experiments.

**Fig. 3.** Effect of CMZ on CYP2E1 apoprotein in Fao cells after CHX treatment. The values of densitometric scanning of Western blots divided by the scanning values of P-450 reductase are compared internally with the control of each experiment (no treatment, 100% constitutive CYP2E1 expression) and are mean ± S.E.M. from three independent experiments. *p < .05 compared with the CHX group. Inset, immunoblot analysis of microsomal proteins isolated from Fao cells treated with CMZ and/or CHX for 6 h. Top, P-450 reductase. Bottom, CYP2E1.

**Discussion**

Our data indicate that CMZ inhibits the constitutive expression of CYP2E1 in Fao rat hepatoma cells and in stably CYP2E1 cDNA transfected V79 cells entirely at a post-trans-
likely that the mode of inhibition on levels (Tindberg and Ingelman-Sundberg, 1996). It appears that the gene is under the control of the SV40 promoter. In the presence of CMZ, the steady-state level of CYP2E1 was reduced and the remaining fraction was still catalytically active.

Under in vivo conditions, it is apparent that CMZ influences the rate of gene transcription as assayed in run-off experiments, with the mRNA levels as well as the protein levels of CYP2E1 determined by Northern blotting and Western blotting, respectively (Hu et al., 1994). A concomitant decrease with treatment of the rats with CMZ was observed at all these levels when the rats had been subjected to starvation for 2 days. In addition, YH439, a thiazolium compound with structures similar to CMZ, was shown to constitute an efficient CYP2E1 inhibitor acting on the transcriptional level (Jeong et al., 1996). By contrast, in the Fao hepatoma cell line, it is evident that CMZ acts post-translationally, as revealed by the decrease in catalytic activity and protein occurring 6 h after CMZ treatment. The effect is half-maximum at 5 μM, which is a relevant concentration in relation to the plasma levels reached during treatment with CMZ; a similar half-maximal effect has been registered in astroglial cultures in which CMZ inhibited the lipopolysaccharide-mediated increase in the expression of CYP2E1 at the mRNA and protein levels (Tindberg and Ingelman-Sundberg, 1996). It appears likely that the mode of inhibition on CYP2E1 expression by CMZ in rat liver requires other important cells, in particular, Kupffer cells. CMZ has been shown to influence the cytokine expression in livers from rats chronically treated with ethanol (Fang et al., 1998). Thus, the increased expression of tumor necrosis factor-α, transforming growth factor-β, and interleukin-1β was inhibited by CMZ; in addition, CMZ has some effects on the cytokine expression seen under control conditions. This action on the cytokine level might be caused by a primary inhibition of the CYP2E1 enzyme, resulting in less oxidative stress and subsequent cytokine release, but it might also be exerted primarily at the level of Kupffer cells, causing alteration in the cytokine release. At present, it is not possible to distinguish the extent to which CMZ acts on the respective levels. Thus, it might be plausible that the major differences observed between the in vitro system used here based on rat hepatoma cells and the in vivo results are accounted for by the lack of a CMZ effect on the cytokine levels in the in vitro system that is otherwise important for the transcriptional regulation of CYP2E1 gene. It is known that interleukin-4, for example, has a pronounced stimulatory effect on the hepatic CYP2E1 expression (Abdel-Razzak et al., 1993). However, other explanations could not be ruled out. It could be plausible, for instance, that both in the in vivo starvation model and in the astroglial cultures mentioned above (Hu et al., 1994; Tindberg and Ingelman-Sundberg, 1996), an early post-translational effect is not reported due to the experimental set-up designed for long-term studies of ethanol or endotoxin treatment. In both cases, inhibition of CYP2E1 transcription by CMZ was reported after a 2-day starvation period or a 24-h lipopolysaccharide treatment, respectively. Thus, in fact, one should not rule out the possibility that the post-translational effect proceeds to the transcriptional one and that both mechanisms are used by the drug, resulting in the potent and prolonged inhibition seen in vivo.

It is interesting to note that a change of medium of the Fao cells caused a rapid increase in the rate of CYP2E1 gene expression as monitored by quantification of the hRNA levels (Fig. 2). This appears to be the first documented hepatic in vitro system in which it is possible to study the transcriptional regulation of this gene. The factors influencing the CYP2E1 transcriptional activity are, of course, unknown, but it might be speculated that extracellular receptors are important in the signal transduction process mediating the stimuli from components in the medium and/or serum. In astroglial cultures, it has been shown that endotoxin stimulates CYP2E1 expression in a process sensitive to inhibitors of tyrosine kinases, indicating the participation of important growth-stimulatory tyrosine kinase-mediated pathways in this event (Tindberg and Ingelman-Sundberg, 1996). It will be interesting to evaluate the DNA motifs and mechanisms involved in this transcriptional activation.

The exact mechanism of CMZ-mediated post-translational inhibition of CYP2E1 is not yet clear. It has previously been shown that CMZ does not act as a competitive inhibitor of rat CYP2E1 (Hu et al., 1995). However, the finding of CMZ as an allosteric inhibitor of human CYP2E1 (Gebhardt et al., 1997) indicates a modulatory action on the enzyme itself. It is evident that CYP2E1 covalently modified by phosphorylation is subjected to rapid degradation (Eliasson et al., 1990), and it indicates a modulatory action on the enzyme itself. It is evident that CYP2E1 covalently modified by phosphorylation is subjected to rapid degradation (Eliasson et al., 1990), and it is speculated that extracellular receptors are important in the signal transduction process mediating the stimuli from components in the medium and/or serum. In astroglial cultures, it has been shown that endotoxin stimulates CYP2E1 expression in a process sensitive to inhibitors of tyrosine kinases, indicating the participation of important growth-stimulatory tyrosine kinase-mediated pathways in this event (Tindberg and Ingelman-Sundberg, 1996). It will be interesting to evaluate the DNA motifs and mechanisms involved in this transcriptional activation.
CMZ has a strong inhibitory effect on the expression of human CYP2E1 as monitored in vivo by CZN hydroxylase activity in control subjects and alcohols treated with the drug (Gebhardt et al., 1997). Despite the short half-life of CMZ, the inhibitory effect persisted for a long time after drug withdrawal, which indicates an influence on the transcriptional level. However, the post-translational effect of CMZ documented here indeed emphasizes the double action of CMZ, resulting in a rapid and persistent decline of CYP2E1 in vivo, a fact that must be considered with respect to drug interactions when using CMZ to treat patients.

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CMZ has a strong inhibitory effect on the expression of human CYP2E1 as monitored in vivo by CZN hydroxylase activity in control subjects and alcohols treated with the drug (Gebhardt et al., 1997). Despite the short half-life of CMZ, the inhibitory effect persisted for a long time after drug withdrawal, which indicates an influence on the transcriptional level. However, the post-translational effect of CMZ documented here indeed emphasizes the double action of CMZ, resulting in a rapid and persistent decline of CYP2E1 in vivo, a fact that must be considered with respect to drug interactions when using CMZ to treat patients.

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