Biochemical Pharmacology, Vol. 51, pp. 701–706, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02391-7

Effects of Phenobarbital and Interleukin-6 on Cytochrome P4502B1 and 2B2 in Cultured Rat Hepatocytes

Michelle A. Clark, Joseph F. Williams, Paul E. Gottschall and Lynn Wecker†*

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
UNIVERSITY OF SOUTH FLORIDA, COLLEGE OF MEDICINE, TAMPA, FL 33612, U.S.A.

ABSTRACT. The objectives of this study were to characterize further the effects of phenobarbital (PB) on cytochrome P4502B1 and 2B2 (P4502B1/2) enzyme activity and immunoreactivity in rat hepatocytes and to investigate the mechanism(s) mediating the ability of interleukin-6 (IL-6) to inhibit this induction. PB caused a concentration-dependent increase in benzyloxyresorufin O-deethylase (BROD) activity with maximal effects (a 25-fold increase) at concentrations of 0.3 to 1 mM. The induction of BROD activity was linear over 24 hr of exposure. Immunoblot profiles of P4502B1/2 agreed with measurements of enzyme activity. In addition to inducing P4502B1/2, PB (0.75 mM) also increased the levels of P450 reductase by approximately 2-fold following a 24-hr exposure to PB. When IL-6 was added concomitantly with or up to 12 hr after the addition of PB, the PB induction of BROD activity and immunoreactivity was inhibited significantly. When 18 hr elapsed between the time of addition of PB and IL-6, the inhibitory effects of IL-6 were no longer apparent, suggesting that the actions of IL-6 were mediated by early events in the induction process. IL-6 did not affect the PB induction of P450 reductase. To determine whether IL-6 altered the degradation of P4502B1/2, hepatocytes were exposed to PB for 24 hr, then washed, and the loss of BROD activity and immunoreactivity following incubation with a protein synthesis inhibitor was measured. IL-6 did not alter the rate of loss of either enzyme activity or immunoreactivity, indicating that the effects of IL-6 could not be attributed to the enhanced degradation of P4502B1/2. Results suggest that the inhibition of PB-induced BROD activity by IL-6 is due to an action on early cellular and molecular events in the induction process. BIOCHEM PHARMACOL 51;5:701–706, 1996.

KEY WORDS. cytochrome P4502B1/2; NADPH cytochrome P450 reductase; benzyloxyresorufin O-deethylase; phenobarbital; hepatocytes; interleukin-6

Inflammation or infection initiates a cascade of local and systemic reactions commonly known as the acute phase response [1]. One of the systemic changes observed is a decrease in the levels and activities of P450s,‡ an effect mediated by various cytokines [for review, see Ref. 2]. However, the mechanism(s) mediating this effect has not been elucidated; there are several steps in the synthesis as well as the degradation of the P450s that may be affected by the cytokines.

In vivo studies suggest that cytokines affect primarily the synthesis of P450s. Experimental inflammation, injection of INF inducers, and the administration of IL-1 have been shown to suppress P4502C11 and P4502C12 protein and mRNA levels [3–5]. Other studies have implicated transcriptional or post-transcriptional effects on mRNA levels as the cause for the loss of P450 by the cytokines [6–8]. Also, Abdel-Razzak *et al.* [9] found that IL-6, IL-1β, and TNF-α significantly depress several P450 (P4501A2, 2C, 2E1, and 3A) enzymatic activities and mRNA expression, whereas γ-INF suppresses only P4501A2 and 2E1 mRNA levels. Using cultured rat hepatocytes, Williams *et al.* [10] have shown that IL-6 (50 U/mL) blocks the induction of P4502B1 mRNA levels, whereas Barker *et al.* [11] found that low concentrations of IL-1β cause an 85% inhibition of P4501A1 and 1A2 mRNAs, but that IL-6 is ineffective at all doses studied. However, Fukuda *et al.* [12] reported that IL-6 down-regulates the transcripts encoding P450IA1, IA2 and IIIA3 in human hepatoma cells, supporting an effect of IL-6 on P450 synthesis.

In addition to evidence supporting an effect of the cytokines on the synthesis of P450s, studies also indicate that cytokines may affect the degradation of P450s. The induction of xanthine oxidase by the cytokines and the subsequent production of free radicals, as well as the induction of heme oxygenase, the rate-limiting enzyme in heme degradation, have been suggested to be involved in the destruction of P450s [13–18]. However, these findings have not been supported universally [19–21].

* Deceased April 7, 1994.

† Corresponding author: Lynn Wecker, Ph.D., Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, MDC Box 9, 12901 Bruce B. Downs Boulevard, Tampa, FL 33612–4799. Tel. (813) 974-3823; FAX (813) 974-3081.

Received 31 July 1995; accepted 2 November 1995.

‡ *Abbreviations:* P450, cytochrome P450; INF, interferon; BROD, 7-benzyloxyresorufin O-deethylase; P4502B1/2, P4502B1 and P4502B2; PB, phenobarbital; IL, interleukin; TNF, tumor necrosis factor; LPS, bacterial lipopolysaccharide; and MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

Recent studies from our laboratory [22] have demonstrated that IL-6 and IL-1β inhibit the induction of both BROD activity and P4502B1/2 immunoreactivity by PB, without altering the induction of 7-ethoxyresorufin O-dealkylase activity or P4501A1 or 1A2 immunoreactivity by 3-methylcholanthrene. In addition, the inhibition by the cytokines is concentration dependent with an IC_{50} for IL-6 and IL-1β of 2.0 and 1.6 U/mL culture medium, respectively. Thus, studies suggest that IL-6 inhibits the induction of P4502B1/2 selectively. To elucidate the mechanism(s) mediating this effect, we characterized further the effects of PB and IL-6 on P4502B1/2 enzyme activity and immunoreactivity in hepatocytes and investigated the effects of IL-6 on the degradation of induced P4502B1/2.

MATERIALS AND METHODS

Materials

Human IL-6 (5.1×10^5 U/mg, LPS content < 0.1 pg/U) was provided by the Genetics Institute (Cambridge, MA). Williams' E medium and calcium, magnesium-free Hanks' Balanced Salt Solution were purchased from GIBCO BRL (Grand Island, NY). Collagenase was purchased from the Worthington Biochemical Corp. (Freehold, NJ). Matrigel was bought from Collaborative Research (Bedford, MA). SDS was obtained from Pierce (Rockford, IL), acrylamide from Fisher Scientific (Fair Lawn, NJ) and *N'*,*N'*-methylene bisacrylamide from the Eastman Kodak Co. (Rochester, NY). Dexamethasone sodium phosphate was purchased from Elkin-Sinn Inc. (Cherry Hill, NJ). Resorufin as well as 7-benzyloxyresorufin were purchased from Molecular Probes Inc. (Eugene, OR). All other chemicals were purchased from Bio-Rad Life Sciences (Richmond, CA) or the Sigma Chemical Co. (St. Louis, MO). Dr. M. Adesnick (New York University Medical Center, New York, NY) provided a mouse monoclonal H-8 antibody against P4502B1/2.

Preparation of Hepatocytes

Adult male Fischer 344 rats (200–250 g, Harlan Industries, Indianapolis, IN) were maintained on a 12-hr light/dark cycle and fed a standard diet and water *ad lib*. The animals were allowed at least a week to acclimatize, and hepatocytes were prepared as described using a standard two step-perfusion technique [22, 23]. The viability of the hepatocytes obtained was 85% or greater as determined by trypan blue exclusion. Hepatocytes were resuspended in Williams' E medium that contained ascorbic acid (50 μg/mL), insulin (10 μg/mL), 6 nM selenium, 0.1 μM dexamethasone, and antibiotics (100 U penicillin, 100 μg streptomycin, and 0.25 μg amphotericin B/mL culture medium). The hepatocytes were plated at a density of 3×10^6 cells/6 cm tissue culture plate coated with 120 μL Matrigel (0.32 ± 0.04 mg/mL) and kept at 37° in an atmosphere of 5% CO_2 in air. The medium was changed every 24 hr, and hepatocytes were allowed to recover from the trauma associated with isolation for 48 hr prior to the addition of test compounds. Hepatocytes maintained under these conditions for 72 hr exhibited an approximately 4-fold decreased P4502B1/2 activity as compared with freshly isolated hepatocytes; BROD activity by the former was 8.7 ± 0.6 pmol/min/mg protein, whereas activity by the latter was 34.8 ± 3.2 pmol/min/mg protein. Following exposure to the compounds, the cells were harvested in 20 mM Tris–HCl (pH 7.8), and sonicates were prepared with a Heat Systems W375 Sonicator (Plainview, Long Island, NY).

Enzymatic Assays

The O-deethylation of benzyloxyresorufin was used as a measure of P4502B1/2 activity. BROD activity was determined according to the method of Sinclair *et al.* [23] as described [22]. The product, resorufin, was analyzed fluorometrically (excitation 528 nm and emission 590 nm) on a Perkin-Elmer LS-3B fluorescence spectrometer using authentic resorufin as the standard. Cytochrome P450 reductase activity was determined as described [24, 25]. The assay contained 200 mM Tris-HCl (pH 7.8), 20 μg protein, 10 mM NADPH, 100 mM KCN, and 1.2 mM cytochrome *c* in a total volume of 1 mL. The blank contained all the components of the reaction mixture except NADPH. The reduction of cytochrome *c* was followed spectrophotometrically on a Beckman DU 70 at 25° for 1 min at an absorbance of 550 nm. Enzyme activity was calculated using an extinction coefficient of 21 cm^{-1} mM^{-1}. The protein content of the sonicates was determined by the method of Bradford [26] using bovine serum albumin as the standard. Cell viability was determined using a modification of the MTT assay [27]. Following exposure of hepatocytes to PB and/or cycloheximide, 150 μL MTT (10 mg/mL saline) was added, and samples were incubated for 2 hr at 37°. The cells were lysed and solubilized by the addition of 750 μL of 20% SDS (in 0.02 M HCl) for 6 hr at 37°, and the absorbance of 100-μL aliquots was determined at 570 nm using a Bio-Rad model 450 Microplate Reader. Cell survival was calculated relative to controls.

Immunoblots

For immunoblotting, sonicated samples were separated electrophoretically on a 10%-SDS polyacrylamide gel [23]. The proteins were transferred to nitrocellulose membranes for 1 hr at 100 V. The membranes were blocked for 1 hr with 0.3% Tween-20, washed, and exposed to H-8 mouse monoclonal anti-P4502B1/2 for 1 hr. After extensive washing, the membranes were treated with goat antimouse immunoglobulin conjugated with alkaline phosphatase. The color was developed using 5-bromo-4-chloro-3-indoletoludidine phosphate and *p*-nitroblue tetrazolium chloride. The band intensity was determined using a Bio-Rad Imaging Densitometer GS-670.

RESULTS AND DISCUSSION

PB Induction of P4502B1/2 and NADPH Cytochrome P450 *Reductase*

Experiments in our laboratory demonstrated that IL-6 inhibits the induction of P4502B1/2 in rat hepatocytes 24 hr following the concomitant addition of IL-6 and 2 mM PB [22]. Although

2 mM PB was chosen based on previous studies [10], this concentration produced variability in the data from experiment to experiment, perhaps because optimal conditions were not employed. Although PB has been shown to induce P4502B1/2 in hepatocytes [10, 23, 28, 29], concentration–effect studies are not well documented. Thus, the first series of experiments sought to optimize the effects of PB on the induction of P4502B1/2. The concentration-dependence and time-course of the effects of PB on the induction of BROD activity and the levels of P4502B1/2 immunoreactive protein are shown in Fig. 1. Both BROD activity and P4502B1/2 immunoreactivity exhibited an inverse U-shape as a function of PB concentration with optimal concentrations of PB ranging from 0.3 to 1.0 mM (Fig. 1A). This optimum for inducing

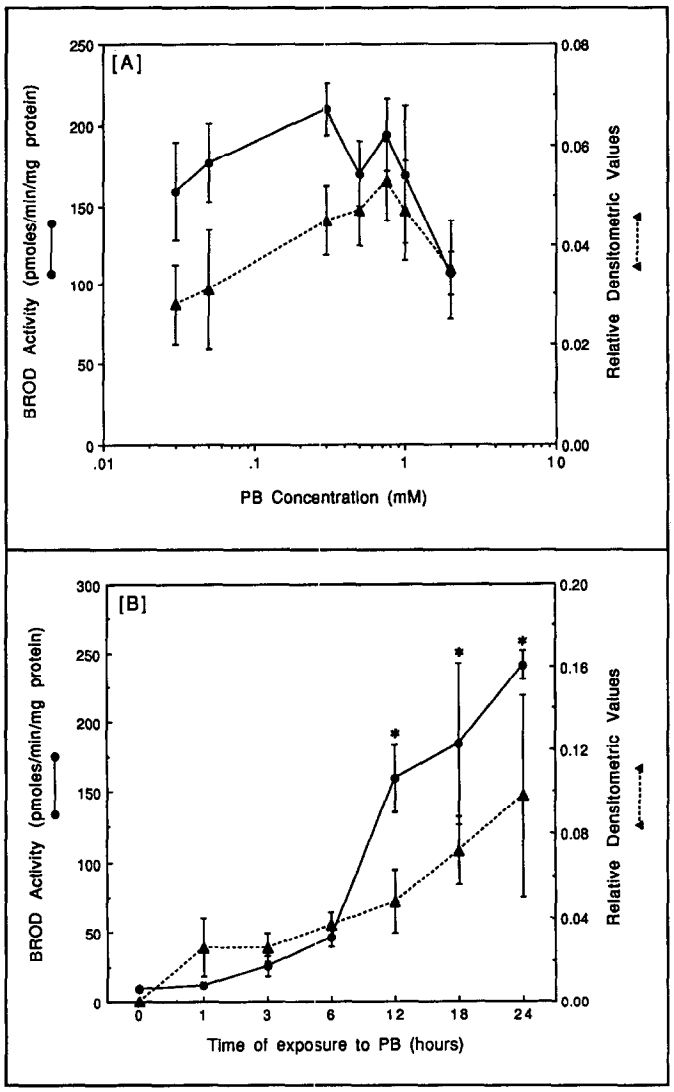

FIG. 1. PB induction of P4502B1/2. Hepatocytes were isolated, processed, and incubated with various concentrations of PB (0.03 to 2 mM) for 24 h [A] or with 0.75 mM PB for 1–24 h [B]. Cells were harvested and sonicated, and BROD activity and immunoreactivity were determined. Each point is the mean ± SEM of 3–4 experiments. Basal values of BROD activity were 8.1 ± 1.0 pmol/min/mg protein. The asterisks denote a significantly ($P < 0.05$) increased BROD activity relative to hepatocytes incubated in the absence of PB.

BROD activity and P4502B1/2 immunoreactivity was similar to the 0.75 mM PB optimum to induce diazepam-metabolizing activity in isolated hepatocytes [30]. The inverse U-shape curve as a function of PB concentration has not been demonstrated previously and suggests that studies in hepatocytes measuring P4502B1/2 that use less than 0.3 mM or more than 1.0 mM PB are being performed under less than optimal conditions.

On the basis of the concentration–response studies, 0.75 mM PB was chosen to determine the time-course of the effect. Hepatocytes were exposed to 0.75 mM PB for up to 24 hr, and BROD activity and P4502B1/2 immunoreactivity were determined (Fig. 1B). Results from these experiments indicate that BROD activity increased slowly during the first 6 hr of exposure to PB, followed by a more rapid increase in activity from 6–24 hr. BROD activity was significantly ($P < 0.05$) different from control (0 time, no PB) values following 12, 18 and 24 hr of exposure; immunoreactivity increased in a similar fashion. The approximately 25-fold increased BROD activity following 24 hr of incubation with 0.75 mM PB is in the range observed for PB induction of P4502B1/2 *in vivo*.

In addition to inducing P4502B1/2, PB has also been shown to induce NADPH cytochrome P450 reductase, the enzyme catalyzing the rate-limiting step in P450-mediated oxidations. Although PB induction of P450 reductase has been demonstrated *in vivo* [31, 32] as well as in hepatocytes isolated from PB-treated rats [33], the ability of PB to induce cytochrome P450 reductase in cultured rat hepatocytes is not well established. Thus, cells were exposed to control medium or medium containing 0.75 mM PB for 24 hr and P450 reductase activity was determined. Results (Table 1) indicate that PB increased P450 reductase activity significantly ($P < 0.05$) by approximately 2-fold. This increase in reductase activity in hepatocytes cultured on Matrigel was similar to the 3 to 4-fold induction of cytochrome P450 reductase by PB in hepatocytes cultured on Vitrogen for 96 hr [30], indicating that Matrigel, like Vitrogen, supports the induction of both P4502B1/2 and NADPH cytochrome P450 reductase.

Inhibition of PB Induction by IL-6

Although IL-6 is well established to inhibit the PB induction of P4502B1/2 [10, 22], the mechanism(s) responsible has not

TABLE 1. Effects of PB and IL-6 on P450 reductase activity in rat hepatocytes

Incubation condition	P450 reductase activity (nmole/min/mg protein)
No drug	19.1 ± 2.60 (N = 6)
PB (0.75 mM)	37.5 ± 2.24* (N = 3)
PB (0.75 mM) + IL-6 (25 U/mL)	39.1 ± 3.38* (N = 3)

Hepatocytes were incubated for 24 hr in the absence or presence of PB. Sonicates were prepared and P450 reductase activity was determined as described in Materials and Methods. Values are means ± SEM.

* Significantly different ($P < 0.05$) from baseline (no drug) values.

been established, i.e. studies have indicated that the cytokines may affect the synthesis as well as the degradation of P450s. To further characterize the effects of IL-6 on PB induction of P4502B1/2, hepatocytes were exposed to PB, and IL-6 (25 U/mL culture medium) was added concomitantly with or 1, 3, 6, 12 or 18 hr after PB. The cells were harvested 24 hr after the addition of PB. Results from these experiments are shown in Fig. 2. When IL-6 was added concomitantly with or up to 12 hr after PB, a significant ($P < 0.05$) decrease in both BROD activity (Fig. 2A) and P4502B1/2 immunoreactivity (Fig. 2B) was observed compared with hepatocytes exposed to PB alone for 24 hr. When IL-6 was added to the hepatocytes 18 hr after PB, the IL-6-mediated inhibition of BROD activity and immunoreactivity was no longer apparent, suggesting that IL-6 may be affecting the synthesis of P4502B1/2. However, these results are confounded somewhat by the time of exposure of the hepatocytes to IL-6, i.e. at the 18-hr interval, hepatocytes

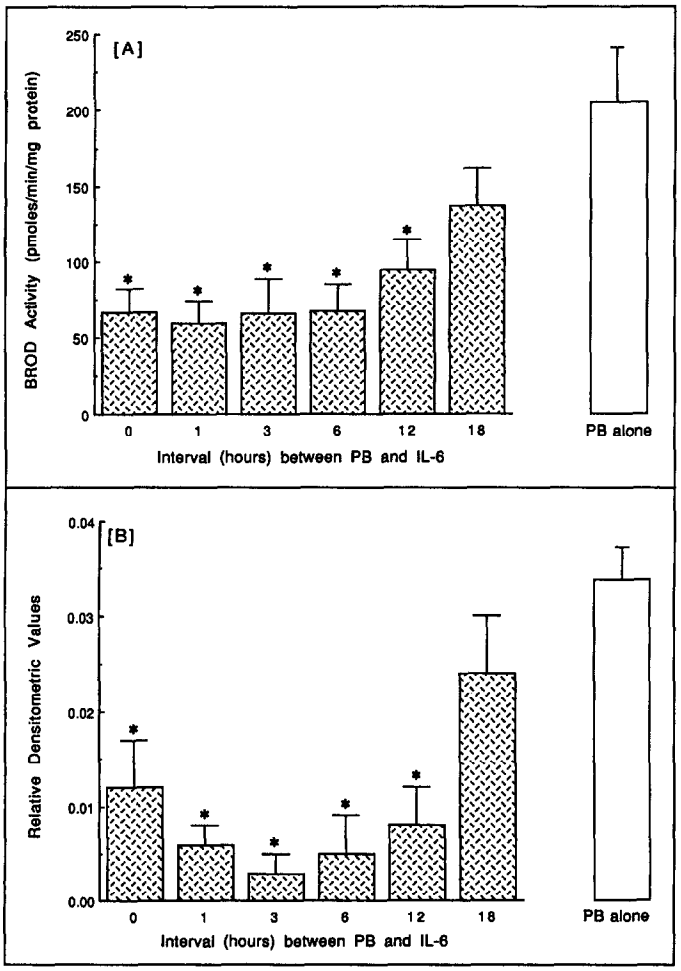

FIG. 2. Time-dependent effects of IL-6 on PB-induced P4502B1/2. Hepatocytes were isolated, processed, and incubated with PB (0.75 mM). IL-6 (25 U/mL culture medium) was added concomitantly with or 1–18 hr after the addition of PB. Cells were harvested 24 hr after the addition of PB, then sonicated, and BROD activity and immunoreactivity were determined. Each point is the mean ± SEM of 3–4 experiments. The asterisks denote a significant ($P < 0.05$) difference compared with hepatocytes incubated with PB alone.

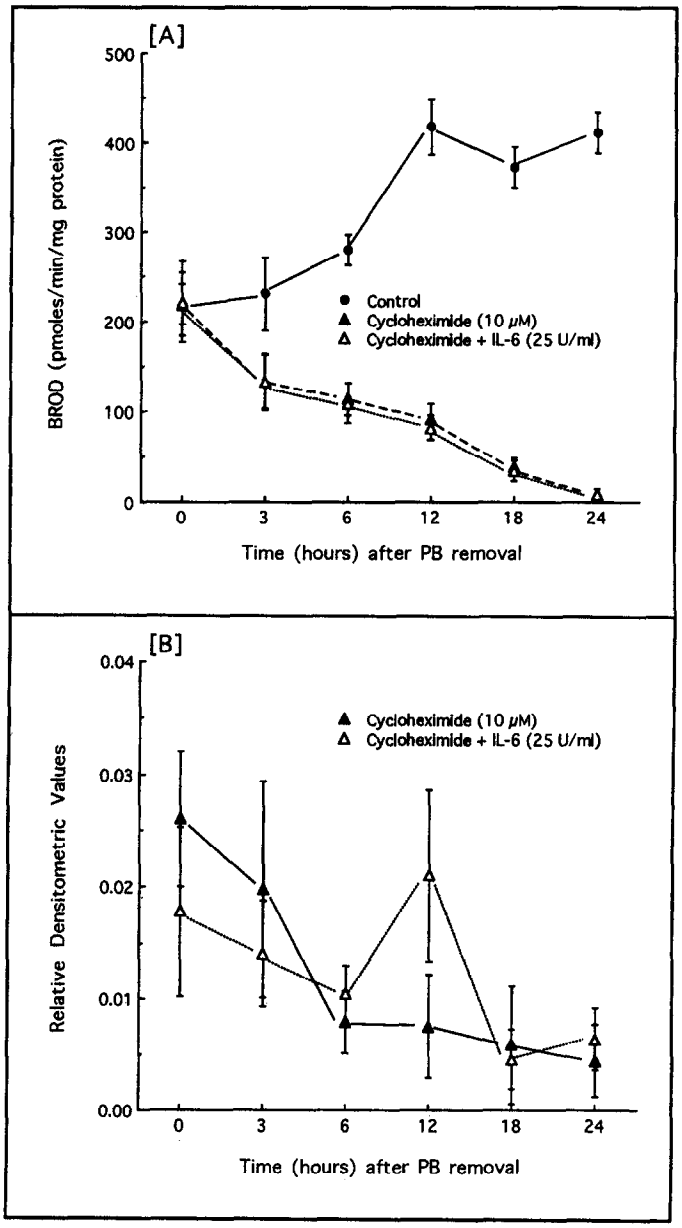

FIG. 3. Effects of IL-6 on the degradation of PB-induced P4502B1/2. Hepatocytes were isolated, processed, and incubated with 0.75 mM PB for 24 hr. Following washing, hepatocytes were incubated with buffer, cycloheximide (10 μM), or cycloheximide (10 μM) and IL-6 (25 U/mL). Cells were harvested at various times, then sonicated, and BROD activity and immunoreactivity were determined. Each value is the mean ± SEM of 4 experiments.

were exposed to IL-6 for only 6 hr, whereas at the 0 time interval, hepatocytes were exposed to IL-6 for 24 hr. Thus, one cannot rule out the possibility that IL-6 promotes enzyme degradation. IL-6 did not alter the PB induction of P450 reductase (Table 1).

To examine the possibility that IL-6 affects enzyme degradation, the effects of IL-6 on the loss of BROD activity and P4502B1/2 immunoreactivity in induced hepatocytes in the presence of a protein synthesis inhibitor were determined. We chose this approach to measure the effect of IL-6 on enzyme

degradation because studies investigating the induction of the enzymes essential in the breakdown of cytochrome P450 (xanthine oxidase and heme oxygenase) have provided equivocal results [18–21, 34]. For these experiments, hepatocytes were exposed to PB for 24 hr and washed prior to the addition of control buffer or cycloheximide (10 μM) in the absence or presence of IL-6 for 3–24 hr; the loss of BROD activity and immunoreactivity was determined. Results from these experiments are shown in Fig. 3. When hepatocytes were exposed to PB for 24 hr, washed, and incubated in control buffer, BROD activity increased steadily during the following 12 hr after which time a maximal effect was achieved. As expected, cycloheximide led to a loss of BROD activity (Fig. 3A) and immunoreactivity (Fig. 3B), both of which decreased to undetectable values by 24 hr. These decreases could not be attributed to nonspecific cytotoxic effects of cycloheximide because cell viability, as assessed by the MTT assay, was unaltered by the presence of cycloheximide; the optical density values for control, PB-treated, and PB-treated preparations in the presence of cycloheximide were 0.89 ± 0.2, 0.90 ± 0.4, and 0.88 ± 0.05, respectively.

IL-6 had no effect on the loss of enzyme activity or immunoreactivity as compared with cycloheximide alone. If IL-6 increased the degradation of P4502B1/2 via activation of an enzyme, one would expect to see a more rapid decline in P4502B1/2 activity or immunoreactivity. Results suggest that IL-6 inhibits P450 induction via events involved in enzyme synthesis. This idea is supported by results indicating that when BROD activity was determined in hepatocytes exposed to IL-6 for 12 hr prior to PB, the PB induction of P4502B1/2 was attenuated. BROD activity in hepatocytes exposed to PB alone for 12 hr was 147 ± 13 pmol/min/mg protein, whereas activity in hepatocytes exposed to IL-6 for 12 hr, followed by washing and incubation with PB for 12 hr was 70.8 ± 5.8 pmol/min/mg protein. Thus, although we cannot rule out degradation completely as it is possible that IL-6 induces another protein that alters P450 degradation, results strongly suggest that IL-6 inhibits P4502B1/2 mRNA expression. Studies are underway to determine the concentration-dependent inhibition of P4502B1/2 mRNA levels as well as the effects of IL-6 on the half-life of P4502B1/2 mRNA.

This study was supported by USPHS/NIH Grant GM47629. The authors thank Ms. Bettye A. Bing for her technical assistance.

References

1. Heinrich PC, Castell JV and Andus T, Interleukin-6 and the acute phase response. *Biochem J* **265:** 621–636, 1990.
2. Williams JF and Szentivanyi A, Pharmacokinetic and pharmacodynamic parameters affected by RE cell activators. In: *The Reticuloendothelial System. A Comprehensive Treatise* (Eds. Hadden JW and Szentivanyi A), Vol. 8, pp. 1–25. Plenum Press, New York, 1985.
3. Morgan ET, Suppression of constitutive cytochrome P-450 gene expression in livers of rats undergoing an acute phase response to endotoxin. *Mol Pharmacol* **36:** 699–707, 1989.
4. Morgan ET and Norman CA, Pretranslational suppression of cytochrome P-450h (IIC11) gene expression in rat liver after administration of interferon inducers. *Drug Metab Dispos* **18:** 649–653, 1990.
5. Wright K and Morgan ET, Regulation of cytochrome P450IIC12 expression by interleukin-1α, interleukin-6, and dexamethasone. *Mol Pharmacol* **39:** 468–474, 1991.
6. Stanley LA, Adams DJ, Lindsay R, Meehan RR, Liao W and Wolf CR, Potentiation and suppression of mouse liver cytochrome P-450 isozymes during the acute phase response induced by bacterial endotoxin. *Eur J Biochem* **174:** 31–36, 1988.
7. Wright K and Morgan ET, Transcriptional and post-transcriptional suppression of P4502C11 and P450IIC12 by inflammation. *FEBS Lett* **271:** 59–61, 1990.
8. Renton KW and Knickle LC, Regulation of hepatic cytochrome P-450 during infectious disease. *Can J Physiol Pharmacol* **68:** 777–781, 1990.
9. Abdel-Razzak Z, Loyer P, Fautrel A, Gautier J-C, Corcos L, Turlin B, Beaune P and Guillouzo A, Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* **44:** 707–715, 1993.
10. Williams JF, Bement WJ, Sinclair JF and Sinclair PR, Effects of interleukin 6 on phenobarbital induction of cytochrome P-450IIB in cultured rat hepatocytes. *Biochem Biophys Res Commun* **178:** 1049–1055, 1991.
11. Barker CW, Fagan JB and Pasco DS, Interleukin-1β suppresses the induction of P4501A1 and P4501A2 mRNAs in isolated hepatocytes. *J Biol Chem* **267:** 8050–8055, 1992.
12. Fukuda Y, Ishida N, Noguchi T, Kappas A and Sassa S, Interleukin-6 down regulates the expression of transcripts encoding cytochrome P450 IA1, IA2 and IIIA3 in human hepatoma cells. *Biochem Biophys Res Commun* **184:** 960–965, 1992.
13. Deloria L, Abbot V, Gooderham N and Mannering GJ, Induction of xanthine oxidase and depression of cytochrome P450 by interferon inducers: Genetic difference in the responses of mice. *Biochem Biophys Res Commun* **131:** 109–114, 1985.
14. Koizumi A, Hasegawa L, Rodgers KE, Ellefson D, Walford RL and Imamura T, Influence of H-2 haplotypes on poly IC induction of xanthine oxidase and poly IC induced decreases in P-450 mediated enzyme activities. *Biochem Biophys Res Commun* **138:** 246–253, 1986.
15. Moochhala SM and Renton KW, A role for xanthine oxidase in the loss of cytochrome P-450 evoked by interferon. *Can J Physiol Pharmacol* **69:** 944–950, 1991.
16. Bissell DM and Hammaker LE, Cytochrome P-450 heme and the regulation of hepatic heme oxygenase activity. *Arch Biochem Biophys* **176:** 91–102, 1976.
17. Gemsa D, Woo CH, Fudenberg HH and Schmid R, Stimulation of heme oxygenase in macrophages and liver by endotoxin. *J Clin Invest* **53:** 647–651, 1974.
18. Cantoni L, Rossi C, Rizzardini M, Gadina M and Ghezzi P, Interleukin-1 and tumor necrosis factor induce hepatic haem oxygenase. Feedback regulation by glucocorticoids. *Biochem J* **279:** 891–894, 1991.
19. Mannering GJ, Deloria LB and Abbot V, Role of xanthine oxidase in the interferon-mediated depression of the hepatic cytochrome P-450 system in mice. *Cancer Res* **48:** 2107–2112, 1988.
20. Cribb AE and Renton KW, Dissociation of xanthine oxidase induction and cytochrome P450 depression during interferon induction in the rat. *Biochem Pharmacol* **46:** 2114–2117, 1993.
21. Cantoni L, Carelli M, Ghezzi P, Delgado R, Faggioni R and Rizzardini M, Mechanisms of interleukin-2-induced depression of hepatic cytochrome P-450 in mice. *Eur J Pharmacol* **292:** 257–263, 1995.
22. Clark MA, Bing BA, Gottschall PE and Williams JF, Differential effect of cytokines on the phenobarbital or 3-methylcholanthrene induction of P450 mediated monooxygenase activity in cultured rat hepatocytes. *Biochem Pharmacol* **49:** 97–104, 1995.
23. Sinclair PR, Bement WJ, Haugen SA, Sinclair JF and Guzelian

PS, Induction of cytochrome P-450 and 5-aminolevulinate synthase activities in cultured rat hepatocytes. *Cancer Res* **50:** 5219–5224, 1990.
24. Phillips AH and Langdon RG, Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: Isolation, characterization, and kinetic studies. *J Biol Chem* **237:** 2652–2660, 1962.
25. Guengerich FP, Analysis and characterization of enzymes. In: *Principles and Methods of Toxicology* (Ed. Hayes WA), Vol. 1, pp. 777–814. Raven Press, New York, 1989.
26. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248–254, 1976.
27. Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. *J Immunol Methods* **65:** 55–63, 1983.
28. Waxman DJ and Azaroff L, Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J* **281:** 577–592, 1992.
29. Schuetz EG, Li D, Omiecinski CJ, Muller-Eberhard U, Kleinman HK, Elswick B and Guzelian PS, Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. *J Cell Physiol* **134:** 309–323, 1988.
30. Waxman DJ, Morrissey JJ, Naik S and Jauregui HO, Phenobarbital induction of cytochrome P450. High-level long-term responsiveness of primary rat hepatocyte cultures to drug induction, and glucocorticoid dependence of the phenobarbital response. *Biochem J* **271:** 113–119, 1990.
31. Shephard EA, Phillips IR, Pike SF, Ashworth A and Rabin BR, Differential effect of phenobarbital and β-napthoflavone on the mRNAs coding for cytochrome P450 and NADPH cytochrome P450 reductase. *FEBS Lett* **150:** 375–380, 1982.
32. Bagley DM and Hayes JR, Neonatal phenobarbital administration results in increased cytochrome P450-dependent monooxygenase activity in adult male and female rats. *Biochem Biophys Res Commun* **114:** 1132–1137, 1983.
33. Utley WS and Mehendale HM, Phenobarbital-induced cytosolic cytoprotective mechanisms that offset increases in NADPH cytochrome P450 reductase activity in menadione-mediated cytotoxicity. *Toxicol Appl Pharmacol* **99:** 323–333, 1989.
34. Fukuda Y and Sassa S, Suppression of cytochrome P4501A1 by interleukin-6 in human HepG2 hepatoma cells. *Biochem Pharmacol* **47:** 1187–1195, 1994.