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Role of Nitric Oxide in the Cytokine-Mediated Regulation of Cytochrome P-450

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SUMMARY

We explored the effects of cytokines on cytochrome P-450 (CYP) in rat hepatocyte primary cultures. CYP content and several CYP protein levels were assessed in hepatocytes treated with a cytokine combination consisting of tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interferon-γ (IFNγ). The combination was found to depress CYP content by 69 ± 6%. Protein levels of CYP forms 1A2, 2C11, 2B1/2, and 3A2 were assessed with immunoblotting. Treatment with the cytokine combination resulted in a decrease in each CYP enzyme, with CYP2B1/2 exhibiting the greatest loss, to 33 ± 9% of untreated cells. The addition of inhibitors of nitric oxide synthase (NOS) significantly prevented the cytokine-mediated decrease in each CYP protein, indicating a role for nitric oxide (NO) in the down-regulation. Treatment of hepatocytes with the NO donor 1-hydroxy-2-oxo-3,3-bis(3-aminoethyl)-1-triazene (300 μM) caused a decrease in each CYP apoprotein, with CYP2B1/2 exhibiting the greatest decrease, to 33 ± 8% of untreated cells. Decreases in CYP protein levels were observed in response to treatment with TNFα, IL-1β, or IL-6 alone. With IL-1β treatment, increased levels of NO production were accompanied by decreased levels of each CYP protein. With TNFα treatment, increased levels of NO production were accompanied by decreased levels of CYP2B1/2 and CYP3A2. The effects of IL-1β and TNFα were blocked by the inclusion of the NOS inhibitors. Conversely, IL-6 caused a decrease in each of the CYP enzymes but did not affect NO production. The results indicate a dissociation *in vitro* between NOS induction and CYP down-regulation for IL-6 treatment, whereas the down-regulation of CYP by TNFα and IL-1β *in vitro* is directly associated with NO production.

CYPs are a major class of heme-containing proteins that catalyze the oxidative metabolism of endogenous and exogenous substances. CYP enzymes are responsible for the bioactivation and/or detoxification and clearance of many drugs and toxicants, thereby playing a critical role in the pharmacological and toxicological effects of many compounds. Immunological and inflammatory stimuli depress the CYP-mediated hepatic metabolism of a variety of drugs, and decreased CYP activity and content have been observed in animals after infection with bacteria and viruses (1, 2). There is a large body of data that show that immune cells secrete proteins called cytokines that are responsible for the depression of CYP content (for a review, see Ref. 3).

The cytokines IL-1β, IL-6, and TNFα are key endogenous mediators of the hepatic acute phase response, and each has been shown to decrease CYP levels *in vivo* and *in vitro* (4–9). Each of these cytokines has specific receptors on both hepatocytes and nonparenchymal liver cells. For example, TNFα has been demonstrated in rats *in vivo* to depress total CYP by 30% (10). IL-1 has been shown to depress total CYP concentration (11) and activity (5) *in vivo*. In addition, Ferrari *et al.* (12) reported a decrease in CYP1A, CYP3A, CYP2C11, CYP2A1, and CYP2B1/2 activities in fetal rat hepatocytes treated with IL-1β. Chen *et al.* (13) showed that in rats, large doses of IL-6 decrease total CYP by 28%, with varying degrees of decreases in several CYP activities. *In vitro*, IL-6 treatment of rat hepatocytes blocks the phenobarbital induction of CYP2B (14). Despite widespread acceptance that the immune system (and cytokines, in particular) can inhibit drug metabolism, the mechanism of this effect is largely unknown.

Cytokines are also known to induce NOS in hepatocytes (15, 16). NO plays important bioregulatory roles in a number of physiological processes, such as the control of blood pressure, neurotransmission, platelet aggregation, and the cytostatic and cytotoxic action of macrophages. Also, it has been suggested that NO has effects on the CYP system; it has been demonstrated that NO inhibits the CYP-catalyzed *O*-dealkylation of benzyloxyresorufin and ethoxyresorufin *in vitro* (17). Khatsenko *et al.* (18) demonstrated that NO was in-

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ABBREVIATIONS: CYP, cytochrome P-450; TNFα, tumor necrosis factor-α; IL-1β, interleukin-1β; IFNγ, interferon-γ; IL-6, interleukin-6; NOS, nitric oxide synthase; NO, nitric oxide; DETA/NONOate, 1-hydroxy-2-oxo-3,3-bis(3-aminoethyl)-1-triazene; L-NMA, N^G-monomethyl-L-arginine; L-NAME, N^ω-nitro-L-arginine methyl ester; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ECL, enhanced chemiluminescence.

volved not only in the inhibition of CYP activity but also in the decrease in CYP content caused by the immunostimulant lipopolysaccharide *in vivo*. In addition, NO has been implicated in the suppression of induction of CYP1A1 protein and mRNA by β-naphthoflavone in rat hepatocytes (19). These results suggest an important role for NO in the regulation of CYP. However, it is not known whether NO has a role in the cytokine-mediated suppression of constitutive CYP levels. Furthermore, there is no information regarding the effect of the direct addition of NO on CYP levels.

The objectives of the current study were to determine the effect of cytokines on specific constitutive CYP isozyme levels in cultured rat hepatocytes and to assess the involvement of NO in these effects. We used purified TNFα, IL-1β, IFNγ, and IL-6 and determined their effects on NOS and constitutive levels of CYP1A2, CYP2B, CYP2C11, and CYP3A2. We also assessed the effects of the direct addition of an NO donor on these CYP proteins. Our results suggest an important role for NO in mediating the down-regulation of CYP by IL-1β and TNFα but not in the down-regulation caused by IL-6.

Materials and Methods

Reagents. Recombinant human TNFα (specific activity, 6.27×10^4 units/μg) was supplied by Genentech (South San Francisco, CA). Recombinant rat IFN-γ (specific activity, 4×10^3 units/μg) was purchased from GIBCO-BRL (Gaithersburg, MD). Recombinant murine IL-1β (specific activity, 1×10^5 units/μg) and recombinant murine IL-6 were purchased from R and D Systems (Minneapolis, MN). The biological activity of IL-6 (molecular mass, 20.6 kDa) was measured by the manufacturer in a cell proliferation assay with the use of the murine factor-dependent plasmacytoma cell line T1165.85.2.1. The ED_{50} for this effect was 0.05–0.02 ng/ml (catalog No. 406-ML, R and D Systems). L-NMA was purchased from Calbiochem (San Diego, CA). ECL reagent and Hyperfilm-ECL were purchased from Amersham (Arlington Heights, IL). Monoclonal antibodies to CYP1A1, CYP2B1/2, CYP2C11, and CYP3A2 were supplied by Dr. Paul Thomas (College of Pharmacy, Rutgers University, Piscataway, NJ). Peroxidase-conjugated goat IgG fraction to mouse IgG was purchased from Cappel (West Chester, PA). DETA/NONOate was purchased from Alexis (San Diego, CA). All other reagents and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher (Pittsburgh, PA).

Cell isolation, culture, and treatment. Hepatocytes were isolated from adult male Sprague-Dawley rats (250–350 g) by collagenase perfusion (20) and were purified by differential centrifugation. Select cell preparations were centrifuged through a 37% Percoll gradient at 500 × *g* for further purification. Cell preparations were ≥95% viable with the use of trypan blue exclusion. Hepatocytes were plated onto collagen-coated culture plates in Williams' E media, which contained 10 mM HEPES, pH 7.4, 1.25 mg/ml bovine serum albumin, 6.25 μg/ml insulin, 100 mg/ml streptomycin, and 60 mg/ml penicillin. Cells were plated at a density of 1×10^5 cells/cm^2 and incubated in a humidified atmosphere of 95% air/5% CO_2 at 37° for 3 hr. Nonadherent cells were removed by washing the plates with phosphate-buffered saline, pH 7.4, and cultures were treated with cytokines, NO donor, and/or NOS inhibitors in fresh media. When used, NOS inhibitors were present throughout the incubations at a concentration of 500 μM. Cells were incubated in the presence of cytokines or DETA/NONOate (NO donor) for 24 hr. The doses for the cytokine combination treatment were 0.1 μg/ml TNFα, 100 units/ml IFN-γ, and 100 units/ml IL-1β. The doses for treatments with individual cytokines and DETA/NONOate are given in figure legends.

At the end of the 24-hr incubation, aliquots of media were removed for analysis of nitrite/nitrate levels (see below). The remaining media were discarded, and the cells were scraped from the plate with a rubber policeman into 100 mM potassium phosphate, pH 7.4, and homogenized. Microsomes were prepared from the homogenate with the use of differential centrifugation (21). The pellet from the final 100,000 × *g* spin was homogenized in 100 mM potassium phosphate, pH 7.4, 0.1 mM EDTA, and 20% glycerol. Protein content was determined according to the method of Lowry (22), and CYP concentration was measured with the use of reduced, carbon monoxide-bound versus carbon monoxide-unbound difference spectra (23). The culture conditions, as described, maintained total CYP content for the 24-hr period at ~75% of the level in cells at time 0.

Analysis of Kupffer cell contamination. Flow cytometry and peroxidase staining were used to estimate the amount of Kupffer cells in the hepatocyte preparations. Flow cytometry was performed on freshly prepared hepatocytes through separation of the cells with an EPICS V Coulter cell sorter, excitation at 488 nm, and analysis with forward angle light scatter. Peroxidase staining was carried out on freshly plated cell preparations as described previously (24). Select hepatocyte preparations were isolated as described above with an additional, final centrifugation spin through a 37% Percoll gradient at 500 × *g*; these cells were also analyzed with flow cytometry and peroxidase staining.

Analysis of nitrite and nitrate concentration. Nitrite levels in the medium were determined colorimetrically with a Molecular Devices Thermomax plate reader. Total nitrite/nitrates were measured by reducing the nitrate to nitrite with NADPH:nitrate reductase followed by reaction with Griess reagent according to previously described methods (25, 26).

Western blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed with microsomes from untreated and cytokine-treated rat hepatocytes. For samples to be probed with anti-CYP2C11, 4 μg of microsomal protein was loaded onto the gel. For samples to be probed with all other antibodies, 25 μg of microsomal protein was loaded. Electrophoretically separated proteins were transferred to nitrocellulose, which was then incubated in 2.5% nonfat dry milk in phosphate-buffered saline, pH 7.4, overnight at 4°. The blocked nitrocellulose was incubated with a monoclonal antibody to a CYP enzyme (0.1 μg antibody/ml) for 2 hr. Three washes were carried out with Tris-buffered saline, pH 7.4, 100 mM NaCl, containing 0.1% Tween 20 (wash buffer). The blot was incubated for 45 min with goat anti-mouse IgG conjugated to peroxidase in wash buffer containing 5% goat serum. After additional washing, the blots were detected with ECL reagent and exposed to Hyperfilm-ECL. Bands were quantified with an imaging densitometer (Bio-Rad).

Statistical Analysis. Comparisons between two groups were made with use of the unpaired Student's *t* test. Comparisons between three or more groups were made with use of one-way analysis of variance.

Results

CYP protein levels and nitrite/nitrate production in response to a cytokine combination. Cultured hepatocytes were treated with the combination of cytokines known to contribute to the hepatic acute phase response, and total CYP content was measured spectrally. The results show that treatment with TNFα/IFN-γ/IL-1β decreases CYP to 31 ± 6% of control levels (Fig. 1). This decrease was largely prevented by the addition of the NOS inhibitors L-NMA and L-NAME. In these cells, the CYP content was 111 ± 15% (L-NMA) and 80 ± 9% (L-NAME) of untreated cells. Either L-NMA or L-NAME treatment alone did not significantly alter CYP content relative to untreated cells.

Levels of specific CYP isoforms were determined in microsomes from the treated cells. Immunoblots from representative experiments for each isoform studied are shown in Fig. 2. Densitometry values and nitrite/nitrate values from all ex-

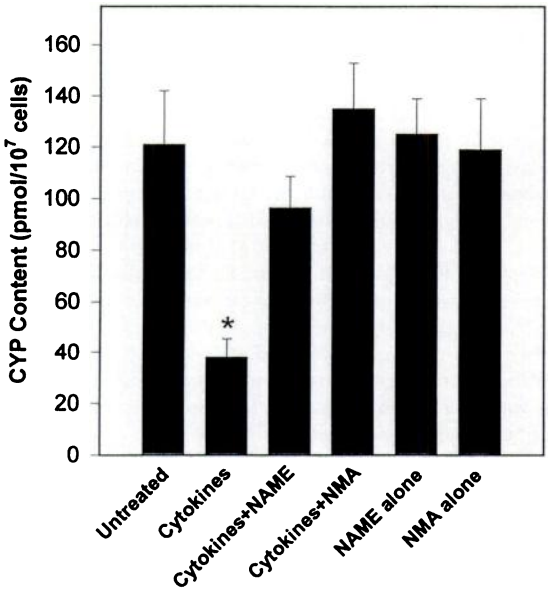

Fig. 1. CYP content in response to treatment with cytokine with and without NOS inhibitor. CYP content was measured with the use of reduced, carbon monoxide-bound versus -unbound difference spectra of the supernatant of a 9000 × *g* spin of the cellular homogenate from cultured hepatocyte samples. Cytokine treatment was a combination of TNFα (0.1 μg/ml), IL-1β (100 units/ml), and IFN-γ (100 units/ml), and the treatment duration was 24 hr. When used, the NOS inhibitors L-NMA (*NMA*) and L-NAME (*NAME*) were present throughout the incubation at 500 μM. *Error bars*, standard deviations from four experiments. *, Significantly different from untreated, $p < 0.05$.

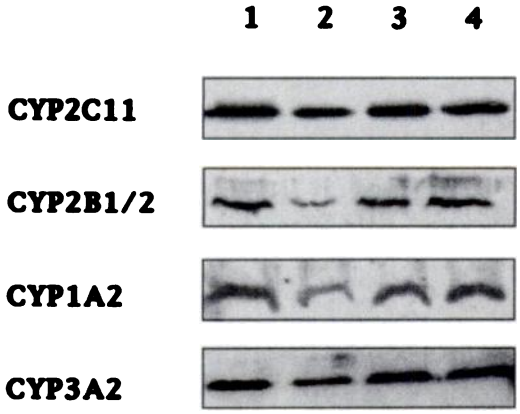

Fig. 2. Immunoblots for CYP forms 2C11, 2B1/2, 1A2, and 3A2 from representative experiments of treatment with cytokine with and without NOS inhibitor. Samples probed were untreated (*lane 1*), cytokine treated (*lane 2*), cytokine treated plus L-NMA (*lane 3*), and cytokine treated plus L-NAME (*lane 4*). Cytokine treatment lasted for 24 hr with the cytokine combination described in legend to Fig. 1. NOS inhibitors, when used, were present throughout the incubation at 500 μM.

periments with the cytokine combination are summarized in Table 1. The 2B antibody that was used recognized both CYP2B1 and CYP2B2, so results are expressed as CYP2B1/2. The hepatocyte cultures were isolated from uninduced rats, so presumably the 2B enzyme detected is predominantly 2B2. Treatment with the cytokine combination results in a decrease in each CYP enzyme, with CYP2B1/2 exhibiting the greatest loss, to 33 ± 9% of untreated cells, and CYP2C11 showing a modest decrease, to 59 ± 7% of untreated cells.

In all experiments, the cytokine combination markedly induced the production of nitrite/nitrate, and as expected, the

TABLE 1

Levels of CYP protein in response to treatment with cytokine with and without NOS inhibitor

Each CYP value represents the percentage of the CYP isoform in untreated cells. Values are mean ± standard deviation from three experiments. Nitrite/nitrate concentration (a measure of NOS activity) was 26 ± 8 μM in media from untreated samples, increased to 249 ± 22 μM in media from cytokine-treated samples, and decreased to 29 ± 8 and 41 ± 12 μM in samples that contained L-NMA and L-NAME, respectively.

CYP isoform	Cytokines[a]	Cytokines + L-NMA	Cytokines + L-NAME
CYP1A2	43 ± 11[b]	78 ± 5[c]	83 ± 7[c]
CYP2B1/2	33 ± 9[b]	91 ± 6[c]	99 ± 8[c]
CYP2C11	59 ± 7[b]	85 ± 8[c]	79 ± 9[c]
CYP3A2	62 ± 4[b]	98 ± 11[c]	96 ± 10[c]

[a] Cytokine combination consisted of 0.1 μg/ml TNFα, 100 units/ml IL-1β, and 100 units/ml IFN-γ.
[b] Significantly different from untreated, $p < 0.05$.
[c] Significantly different from cytokine treated, $p < 0.05$.

production of nitrite/nitrate was completely blocked by the NOS inhibitors. For each CYP protein studied, the NOS inhibitors either partially or completely prevented the cytokine-mediated decrease in the CYP protein levels. NOS inhibitors alone did not affect the levels of any of the CYP proteins relative to control values (data not shown).

Effects of individual cytokines on CYP isoforms and NOS induction. The levels of each CYP protein were decreased by treatment of hepatocytes with IL-1β (Table 2). Treatment with TNFα alone caused a decrease in CYP2B1/2 and CYP3A2 (Table 2). IL-1β treatment caused induction of NOS, and the presence of L-NMA significantly prevented both the production of NO and the down-regulation of each CYP protein. The inclusion of L-NMA also prevented the decrease in CYP2B1/2 caused by TNFα. Treatment of hepatocytes with IL-6 led to a dose-dependent decrease in each of the CYP proteins studied, with CYP2B1/2 and CYP1A2 exhibiting the largest decreases, to levels ~50% of untreated cells (Fig. 3). However, IL-6 treatment did not affect the production of nitrite/nitrate relative to control, indicating that NO is not involved in the effects of IL-6 on CYP protein. In agreement with this observation, the inclusion of L-NMA did not alter the effects of IL-6 on CYP levels (data not shown). The treatment of hepatocytes with IFN-γ alone for 24 hr did not significantly alter the levels of the CYP enzymes studied (data not shown).

Effects of DETA/NONOate treatment on CYP proteins. Treatment of hepatocytes with the NO donor DETA/

TABLE 2

Levels of CYP protein in response to treatment with TNFα or IL-1β with and without L-NMA

Each CYP value represents the percentage of the CYP isoform in untreated cells. Values are mean ± standard deviation from three experiments. Nitrite/nitrate concentration was 22 ± 18 mM in media from untreated samples and 185 ± 12 μM in media from IL-1β-treated samples and decreased to 23 ± 7 μM with IL-1β and L-NMA treatment. Nitrite/nitrate concentration was 112 ± 8 μM in media from TNFα-treated samples and decreased to 33 ± 9 μM with TNFα and L-NMA treatment.

CYP isoform	IL-1β[a]	IL-1β[a] + L-NMA	TNFα[b]	TNFα[b] + L-NMA
CYP1A2	71 ± 9[c]	109 ± 6[d]	83 ± 7	96 ± 8
CYP2B1/2	41 ± 10[c]	111 ± 8[d]	61 ± 9[c]	93 ± 10[d]
CYP2C11	76 ± 7[c]	103 ± 9[d]	94 ± 6	95 ± 4
CYP3A2	54 ± 13[c]	95 ± 12[d]	73 ± 12[c]	89 ± 11

[a] Dose of IL-1β was 100 units/ml.
[b] Dose of TNFα was 1.0 μg/ml.
[c] Significantly different from untreated samples, $p < 0.05$.
[d] Significantly different from cytokine-treated samples, $p < 0.05$.

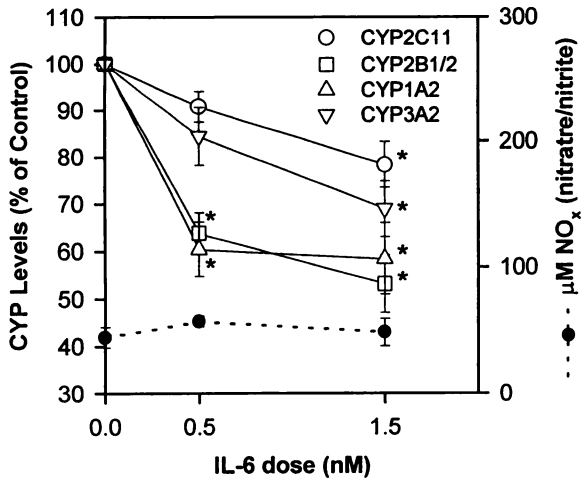

Fig. 3. Levels of CYP apoprotein and nitrite/nitrate production in hepatocytes in response to treatment with IL-6. CYP levels were measured densitometrically. Nitrite/nitrate concentrations were measured spectrophotometrically. *Error bars*, standard deviations from three experiments. *, Significantly different from untreated, $p < 0.05$.

NONOate caused a dose-dependent decrease in each of the CYP proteins (Fig. 4). CYP2B1/2 was decreased to the greatest extent, with 300 μM DETA/NONOate yielding CYP protein levels of 33 ± 7.9% of untreated cells. CYP2C11 showed the least decrease, with a level of 88.4 ± 1.9% of control in response to 300 μM DETA/NONOate.

Validation of hepatocyte-derived NO production. With flow cytometry, it was estimated that the primary hepatocyte preparations contained ~5% nonparenchymal cell contamination. Peroxidase staining indicated that the Kupffer cell contamination was 5–10%. Hepatocytes were then prepared with a final centrifugation spin through a 37% Percoll gradient. These cells contained no detectable Kupffer cell contamination as determined with flow cytometry and contamination of <1% as determined with peroxidase staining. The purified hepatocytes were cultured and incubated with the cytokine combination for 24 hr. Untreated cells yielded nitrite/nitrate concentrations of 12.91 ± 2.78 μM, whereas the cytokine combination-treated cells yielded nitrite/nitrate concentrations of 226.6 ± 17.09 μM. CYP isoform levels were assessed from these cells, and the cytokine treatment resulted in a decrease in CYP levels to values that were within the range of values reported in Table 1: 2B1/2 was decreased to 44% of untreated, 1A2 to 51%, 2C11 to 52%, and 3A2 to 70%.

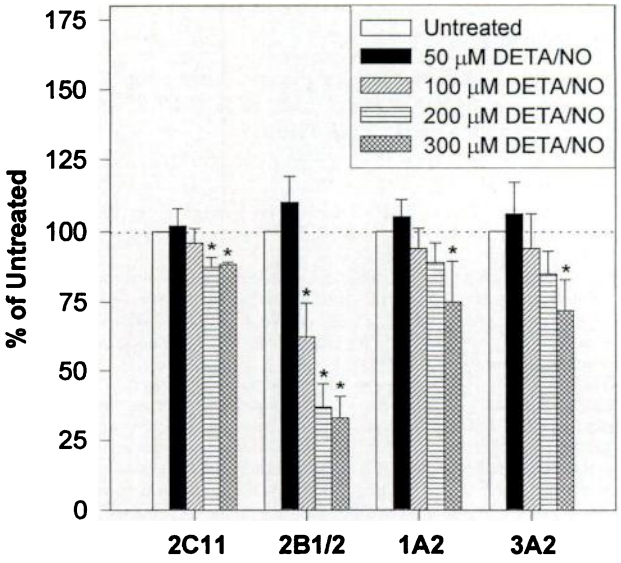

Fig. 4. CYP isoform levels in response to treatment with NO donor. Levels of CYP apoprotein were assessed in microsomes from cells treated with the NO donor DETA/NONOate (*DETA/NO*). CYP levels were measured densitometrically. *Error bars*, standard deviations from four experiments. *, Significantly different from untreated, $p < 0.05$.

Discussion

Inflammation and infectious diseases are characterized by the production of cytokines, which can in turn alter various cellular functions, including drug-metabolizing capacity. The suppression of hepatic drug metabolism by cytokines arises from the reduced activity of the CYP mixed-function oxidase system (1, 3, 4). Suppression of metabolism by CYP diminishes the bioactivation and/or clearance of drugs and toxicants, resulting in profound pharmacological and toxicological effects. In the current study, we examined the effect of direct cytokine treatment on several CYP protein levels in cultured rat hepatocytes. Also, the role of NO in this process was assessed directly by measuring CYP levels in response to treatment with an NO donor. The novel contributions of this work are that (i) cytokine treatment leads to a loss of several constitutive CYP protein levels through an NO-mediated mechanism and (ii) NO addition (via an NO donor) can directly decrease several CYP isoform levels *in vitro*.

A loss of total CYP content was observed in response to treatment with TNFα/IL-1β/IFN-γ. This combination was used because it causes a large induction in NOS in hepatocytes. The loss in CYP content was largely prevented by inclusion of NOS inhibitors in the incubation. To determine whether there was an effect of the cytokine combination treatment on specific CYP enzymes at the level of the apoprotein, we used immunoblotting techniques to examine the levels of four CYP enzymes in response to treatment. The cytokine combination treatment resulted in reduction of each of the isozymes studied (Table 1). CYP2B1/2 was reduced to the greatest extent relative to the decreases in the other CYP enzymes.

Inclusion of NOS inhibitors either partially or completely prevented the decrease in CYP levels, implying that NO is involved in the down-regulation of each of the CYP enzymes studied. The direct addition of DETA/NONOate, which slowly releases NO (27, 28), also caused decreases in CYP. Interestingly, CYP2B was the enzyme studied that was most affected by both the cytokine combination and the NO donor.

NO has been suggested as having a role in CYP effects mediated by cytokines. Khatsenko *et al.* (18) showed that diminished CYP activity in response to *in vivo* lipopolysaccharide treatment was associated with NO overproduction. In addition, Wink *et al.* (17) reported the inhibition of CYP activity in microsomes treated with NO and NO-releasing agents. It has also been reported that a cytokine combination similar to the one we used decreased CYP1A1 and CYP1A2 levels in βNF-treated rat hepatocytes (19). In the induced system, L-NMA protected against the decrease in CYP1A1 levels but did not affect CYP1A2. In the induced hepatocytes, the cytokines and NO may have been acting to either de-

crease CYP levels or prevent the induction of CYP1A. Results of the current study demonstrate NO involvement in the down-regulation of each of the four isoforms studied, including 1A2, in constitutive rat hepatocytes.

In an effort to delineate the role of NO in the effects on CYP elicited by individual cytokines, experiments were performed with TNFα, IL-1β, and IL-6. For treatment with IL-1β alone, increases in the levels of nitrite/nitrate production were accompanied by decreasing levels in each of the CYP enzymes (Table 3). TNFα treatment led to more modest increases in nitrite/nitrate production and significant decreases in CYP2B1/2 and CYP3A2. The effects of IL-1β on each CYP isoform and the effect of TNFα on CYP2B1/2 were blocked by inclusion of the NOS inhibitors in the incubations, indicating a role for NO in mediating the down-regulation of CYP by these cytokines. On the other hand, IL-6 caused a decrease in each of the CYP enzymes while not affecting nitrite/nitrate values (Fig. 4). The doses of IL-6 used in these experiments have also been shown to not induce NOS protein levels in hepatocytes.[1] The inclusion of L-NMA in treatments with IL-6 did not affect the IL-6-mediated effects on CYP. Treatment with IFN-γ alone did not lead to significant alterations in CYP proteins in our *in vitro* system. Interferons have been shown to decrease CYP; however, carefully defined media including steroids, which we did not use, were needed to elicit the effects of interferon on CYP *in vitro* (29, 30).

Finally, we carried out experiments to validate that the NO was being produced in hepatocytes and was not a result of contaminating Kupffer cells in the primary cultures. By preparing hepatocytes with centrifugation through a Percoll gradient, we were able to eliminate detectable Kupffer cells from the hepatocyte preparation. The Percoll gradient-treated hepatocyte preparations yielded results similar to those from typically prepared hepatocytes in response to cytokine combination treatment with regard to both nitrite/nitrate production and CYP decreases. Consistent with these data, cytokines have been shown to induce the mRNA level of NOS in hepatocytes (31), and these increases correlate with increases in NOS protein in hepatocytes and nitrite/nitrate levels in culture medium of hepatocytes treated with cytokines.[1] The results from the current study, taken together with the previous data, demonstrate that hepatocyte-derived NO decreases CYP protein levels.

The results demonstrate that there are at least two mechanisms by which immune stimulation depresses CYP apoprotein levels in cultured rat hepatocytes. Certain cytokines (IL-1β and TNFα) act by inducing NO production, which depresses CYP. Also, the role of NO in CYP down-regulation is supported by the fact that the direct addition of the NO donor DETA/NONOate to hepatocytes depresses CYP protein levels (Fig. 4). On the other hand, IL-6 treatment does not induce NOS, but it does depress CYP levels. Taken together, the results indicate cytokine-dependent mechanisms of CYP down-regulation.

The mechanism of NO-mediated down-regulation of CYP is unknown. NO could impair CYP concentrations and activities at the protein level by enhancing degradation of heme or apoprotein moieties of CYP. It has been shown that NO binds to the heme of CYP enzymes with high affinity (18, 32) and that NO/CYP complexes are relatively unstable (32). Furthermore, it has been recently demonstrated that cellular NO synthesis results in the loss and degradation of enzyme-bound heme (33). Dissociation of the heme from CYP has been postulated as a mechanism for lipopolysaccharide suppression of CYP (18). Alkylation or oxidative damage can also lead to degradation of apoprotein of different CYP enzymes (34). Nitrosylation of CYP enzymes by NO could therefore enhance the degradation of either CYP heme or apoprotein moieties.

Immunostimulants and cytokines have also been shown to diminish steady state mRNA and gene transcription of certain CYP genes (7–9). The degree of cytokine-mediated reduction of CYP mRNA is generally more pronounced than the reduction of the respective protein levels. Abdel-Razzak *et al.* (9) demonstrated marked decreases in mRNA levels of several CYP enzymes in response to individual cytokine treatment in cultured human hepatocytes. Recently, NO was implicated in the transcriptional effects of cytokines on CYP. In βNF-treated rat hepatocytes, L-NMA has been shown to prevent the suppression of CYP1A1 mRNA by cytokines (19). It has been speculated that NO nitrosylates transcriptional factors and thus suppresses transcription of CYP mRNA (18). Various reports indicate that NO can influence protein concentrations at multiple levels of regulation. Further study is required to define the relative role of the effects of NO at each of these levels in elucidating the mechanism of NO-mediated effects on CYP enzymes.

In summary, we have shown that NO is an important mediator of the IL-1β and TNFα down-regulation of several constitutive CYP isoforms in rat hepatocyte culture. We have also shown a dissociation between NO production and CYP suppression caused by IL-6, indicating cytokine-dependent mechanisms of CYP down-regulation. The results reveal basic interactions among cytokines, NO, and metabolism in infectious and inflammatory states and how these phenomena may have an impact on chemical-mediated hepatotoxicity.

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