Microsomal Cytochrome P450 Levels and Activities of Isolated Rat Livers Perfused with Albumin

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Purpose. We recently showed that the perfusion of isolated rat livers with perfusates containing bovine serum albumin (BSA) would significantly stimulate the release of tumor necrosis factor (TNF)-α. Here, we hypothesize that BSA-induced increase in the release of TNF-α, and possibly other cytokines, would affect cytochrome P450 (CYP)-mediated drug metabolism.

Methods. Rat livers were perfused *ex vivo* for 1, 2, or 3 h with a physiologic buffer containing or lacking 1% BSA (n = 4–5/group). At the end of perfusion, liver microsomes were prepared and analyzed for their total CYP, CYP2E1, CYP3A2, and CYP2C11 protein contents and the activities of cytochrome c reductase, CYP2E1, CYP3A2, CYP2C11, CYP2E1, CYP2D1, CYP1A1, and CYP2B1/2. In addition, the concentrations of various cytokines and nitric oxide were quantified in the outlet perfusate.

Results. In the absence of BSA, the perfusate levels of all measured cytokines and nitric oxide were low. However, when the perfusate contained BSA, the levels of TNF-α, interleukin-6, and nitric oxide increased significantly ($p < 0.005$). Perfusion of the livers for 3 h with the BSA-containing perfusate resulted in significant ($p < 0.05$) decreases in the total CYP (41%), CYP2E1 (59%), CYP3A2 (68%), and CYP2C11 (50%) protein contents and activities of cytochrome c reductase (31%), CYP2E1 (66%), CYP3A2 (54%), and CYP2C11 (51%). In contrast, perfusion of livers for 1 or 2 h with the BSA perfusate did not have any significant effect on CYP-mediated metabolism. The CYP1A2, CYP2D1, and CYP2B1/2 activities were not affected by BSA, regardless of perfusion time.

Conclusion. Addition of BSA to perfusates, which is a routine practice in isolated rat liver studies, can reduce CYP-mediated drug metabolism by a mechanism independent of protein-binding effect.

KEY WORDS: liver perfusion; bovine serum albumin; lipopolysaccharide; cytochrome P450; drug metabolism.

INTRODUCTION

Endotoxins or lipopolysaccharides (LPS), components of the outer portion of the cell wall of gram-negative bacteria, suppress hepatic microsomal cytochrome P450 (CYP) enzymes (1). The effect of LPS on CYP levels is indirect and involves activation of Kupffer cells and subsequent release of various cytokines (2). Among the released cytokines, tumor necrosis factor (TNF)-α has been implicated (3,4) as an important mediator of the physiologic effects of LPS. The administration of TNF-α has been shown to decrease total CYP as well as CYP1A, CYP2B, CYP2C, CYP2E, and CYP3A subfamilies (3,4). Furthermore, TNF-α stimulates the production and secretion of other cytokines, including interleukin (IL)-1, IL-6, and interferon-γ (IFN-γ), which have also been shown to down-regulate the same CYP subfamilies (5).

The isolated perfused rat liver (IPRL) model has been used by many investigators to study the metabolism of various drugs (6). The perfusate routinely used by many investigators for drug metabolism studies contains bovine serum albumin (BSA) in a physiologic buffer (6). Bovine serum albumin preparations are added to perfusates either as oncotic agents or for studying the effects of protein binding on drug metabolism and uptake by the liver. Because only the unbound drug can enter hepatocytes, the addition of BSA to perfusates is generally expected to result in a decrease in the hepatic extraction ratio of drugs that are substantially bound to BSA (7,8).

Recently (9), we showed that the addition of some commercially available preparations of BSA to the perfusate would substantially accelerate TNF-α production. Whereas in the absence of BSA, the TNF-α levels remained close to zero, the steady-state levels of TNF-α reached as high as 1200 pg/mL in the presence of BSA (9). The BSA-induced increase in the TNF-α levels of the liver perfusates was attributed to the endotoxin content of the BSA preparations; addition of a very low-endotoxin BSA to the perfusate did not substantially increase the TNF-α levels (9). Because cytokines and other proinflammatory mediators may alter drug metabolism, it is possible that BSA-containing perfusates affect drug metabolism in IPRL by a mechanism independent of protein binding. Therefore, the studies reported here were designed to test the hypothesis that proinflammatory mediators released during the perfusion of livers with the BSA-containing perfusates would affect CYP-mediated drug metabolism.

MATERIALS AND METHODS

Chemicals and Buffers

Bovine serum albumin (BSA, Fraction V, Catalog A-3912), benzyloxyresorufin, testosterone, 6β-hydroxytestosterone, *p*-nitrophenol, *p*-nitrocatechol, dextromethorphan, cytochrome c, $NADP^+$, NADPH, isocitrate dehydrogenase, antigoat rabbit alkaline phosphate conjugate, and antirabbit goat alkaline phosphate conjugates were purchased from Sigma Chemical Co. (St. Louis, MO). Ethoxyresorufin and 16α-hydroxytestosterone were obtained from Molecular Probes, Inc. (Eugene, OR) and Steraloids Inc. (Wilton, NH), respectively. Goat antirat primary antibody for CYP2E1 was from Gentest (Woburn, MA). Rabbit antirat primary antibodies for CYP3A2 and CYP2C11 were purchased from Chemicon International (Temecula, CA) and Affinity Bioreagents Inc. (Golden, CO), respectively. All other reagents were analytical grade and obtained from various commercial sources.

The following buffers were used in our experiments: 100 mM phosphate buffer (PB) with a pH of 7.4; phosphate-buffered (100 mM, pH 7.4) saline (138 mM NaCl plus 2.7 mM KCl) (PBS); TGE buffer containing 50 mM Tris (pH 8), 20% glycerol, and 0.1 mM EDTA; sucrose buffer containing 250 mM sucrose and 50 mM Tris HCl (pH 7.4); 50 mM carbonate–bicarbonate buffer (pH 9.6); and 50 mM Tris-buffered saline

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with 0.1% Tween 20 (TBS). All the buffers or their components were purchased from Sigma.

Animals

All the procedures involving animals used in this study were consistent with the guidelines set by the National Institute of Health (NIH publication 85-23, revised 1985) and approved by our institutional animal committees. Adult male Sprague–Dawley rats (mean ± SD of body weight: 269 ± 14 g) were purchased from a commercial source and housed in a light- and humidity-controlled animal facility at least 2 days before the experiments. The animals had free access to water and food.

Isolation and Perfusion of Livers

The liver isolation and portal vein, hepatic vein, and bile duct cannulation methods were similar to those reported by us before (8,9). The perfusate was a Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 1.2 g/L glucose and 75 mg/L sodium taurocholate with (Albumin) or without (Control) 1% (w/v) BSA. The perfusate was oxygenated with an O_2:CO_2 (95:5) mixture for at least 15 min before it entered the liver. Livers were perfused, in a single-pass manner, with a flow rate of 30 mL/min. To avoid contamination of the apparatus by endotoxin from bacterial growth or other sources, the apparatus was cleaned after each day of use with 10% Clorox solution (The Clorox Company, Oakland, CA), followed by an extensive rinse with deionized water.

A total of 25 IPRLs were used in this study that consisted of six experimental groups. Livers were perfused with either Control or Albumin perfusates for 1, 2, or 3 h. Each experimental group consisted of four livers except for the 3-h Control group, which contained five livers. After isolation, the livers were allowed to stabilize for ~10 min before the start of the experiment. Samples were collected from the outlet at 0, 10, 20, 30, 40, 50, and 60 min for the 1-h groups, at 0, 15, 30, 45, 60, 80, 100, and 120 min for the 2-h groups, and at 0, 30, 60, 90, 120, 150, and 180 min for the 3-h groups. Collected samples were stored at −80°C for further analysis of the cytokine levels. Additional samples were collected from the outlet at the beginning (time zero) and end of perfusion and stored at 4°C for the measurement of transaminase levels within 1 week. Bile samples were also collected in preweighed microcentrifuge tubes at 30-min intervals.

Sample Analysis

The concentrations of TNF-α, IL-6, IL-1β, and IFN-γ in the outlet perfusates were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, CA). The concentration of LPS in BSA was analyzed using a semiquantitative assay based on horseshoe crab lysate (9). The transaminase levels were quantified based on a colorimetric method using a commercial kit from Sigma (Procedure No. 505).

The concentrations of nitric oxide (NO) in the last outlet perfusate samples (1, 2, or 3 h) were measured based on nitrate/nitrite determination using a commercially available kit (Active Motif, Carlsbad, CA) that uses Griess reagent.

Microsomes, Total CYP Content, and Cytochrome c Reductase Activity

Microsomes were prepared according to the established ultracentrifugation methods (10). The final pellet was suspended in 5 mL of TGE buffer and stored at −80°C. Total protein content was measured by Bradford assay (11) using bovine serum albumin as standard. Spectral analysis of total CYP content was performed using the method of Omura and Sato (12). Cytochrome c reductase activity was measured according to minor modifications of a kinetic method reported before (13). Our total reaction volume was 1 mL, consisting of 10 μL of 20 times diluted microsomes, oxidized cytochrome c (5 μM), and NADPH (100 μM) in PB.

Analysis of CYP Isoform Activity

Testosterone Hydroxylation

The activities of CYP3A2 and CYP2C11 were assessed by formation of 6β- and 16α-hydroxytestosterone, respectively, from testosterone based on a modification of a previously published (14) method. Briefly, testosterone (210 μM) was incubated for 30 min at 37°C in a 500 μL total volume incubation mixture containing $NADP^+$ (1 mM), microsomal protein (1 mg/mL), isocitrate dehydrogenase (0.1 U/mL), and isocitrate (10 mM) in PB. The reaction was terminated by the addition of 0.5 mL of 0.6 M perchloric acid. Samples were centrifuged at 7,500 × *g* for 15 min, and the supernatant was directly injected into the HPLC for analysis of the metabolites.

Para-Nitrophenol Hydroxylation

The activity of CYP2E1 was assessed by *p*-nitrophenol assay (15). Briefly, a 2-mL reaction volume (PB) contained microsomal protein (0.75 mg/mL), *p*-nitrophenol (0.1 mM), isocitrate (20 mM), and isocitrate dehydrogenase (0.2 U/mL). The reaction was initiated at 37°C with the addition of $NADP^+$ (2 mM).

Dextromethorphan Demethylation

The activity of CYP2D1 dependent monooxygenase in rat liver microsomes was assessed by employing [O-methyl-^{14}C]dextromethorphan as a substrate (16). Briefly, a stock solution was prepared from labeled (1 μCi/μL) and unlabeled (200 μM) dextromethorphan to give a final concentration of 10 μM (0.2 μCi/mL). The reaction mixture (1 mL in PB) contained microsomal protein (0.5 mg/mL), isocitrate (10 mM), isocitrate dehydrogenase (0.1 U/mL), and 10 μl of the stock solution of dextromethorphan. After the addition of $NADP^+$ (1 mM), the reaction mixture was incubated for 10 min at 37°C. Following extraction of the samples with dichloromethane, a 0.5-mL aliquot of the aqueous portion was diluted with 5 mL of scintillation cocktail and read using a scintillation counter.

Ethoxyresorufin and Benzyloxyresorufin Dealkylation

The dealkylations of ethoxyresorufin and benzyloxyresorufin were used as measures of the activity of CYP1A1 and CYP2B1/2, respectively (17). Briefly, microsomal proteins (0.3 mg/mL) were added to 2 mL of a reaction mixture in a cuvette containing ethoxyresorufin (2.5 μM) or benzyloxyresorufin (2.5 μM) in phosphate-buffered saline. After incubation for 5 min at 37°C, the reaction was started by the addition of NADPH (0.4 M).

Analysis of CYP2E1, CYP3A2, and CYP2C11 Protein Contents

Liver CYP2E1, CYP3A2, and CYP2C11 protein contents were quantified according to an ELISA method reported previously by Snawder and Lipscomb (18). Microsomal proteins used were 0.15 μg for CYP2E1 and CYP2C11 and 1 μg for CYP3A2 analysis. Standards ranged from 25 to 250 fmol per well for the analysis of CYP2E1 and CYP2C11 and from 10 to 50 fmol per well for the analysis of CYP3A2. Isoform protein values were expressed as picomoles per milligram of protein.

Data Analysis

The area under the perfusate concentration–time curve (AUC) of cytokines from time zero to the last sampling time (1, 2, or 3 h) was estimated using a linear trapezoidal rule. The statistical differences among the groups perfused for 1, 2, or 3 h were tested using two-way ANOVA with subsequent Scheffe's *F* test. Within each perfusion time group, comparisons of the two perfusates (Control versus Albumin) were carried out using unpaired, two-tailed *t* test. Statistical significance was defined as a p value < 0.05. Data are reported as mean ± SD.

RESULTS

Cytokine Concentration–Time Profiles

The time courses of the concentrations of TNF-α in the outlet perfusates of different groups of livers perfused for 1, 2, or 3 h are presented in Fig. 1. When the perfusate did not contain BSA (Control), the concentrations of TNF-α in the outlet perfusate were low during the entire period of perfusion, irrespective of the perfusion time. However, when the perfusate contained 1% BSA (Albumin), the concentrations of TNF-α started to rise at ~20 min after the start of the perfusion and reached a plateau at ~1 h (Fig. 1). The AUC values (ng·h/mL) of TNF-α were 0.0252 ± 0.0054 (1-h Control), 0.296 ± 0.009 (1-h Albumin), 0.264 ± 0.126 (2-h Control), 1.50 ± 0.14 (2-h Albumin), 0.0372 ± 0.0459 (3-h Control), and 2.68 ± 0.56 (3-h Albumin). Within each perfusion time group, the TNF-α AUC values for Control livers were significantly ($p < 0.0001$) lower than those for the livers perfused for the same length of time with BSA (Albumin).

The time courses of the concentrations of IL-6 in the outlet perfusates of rats perfused for 3 h are presented in Fig. 2 for both Control and Albumin perfusates. In the absence of BSA in the perfusate, concentrations of IL-6 in the outlet samples were low during the entire period of perfusion. However with BSA, the perfusate concentrations of IL-6 started to rise at ~1.5 h after the start of the perfusion and continued to rise until the last sampling time at 3 h (Fig. 2). The AUC (ng·h/mL) of IL-6 in Albumin group (0.274 ± 0.039) was significantly ($p < 0.005$, unpaired *t* test) higher than that for Control livers (0.143 ± 0.053).

In contrast to the TNF-α and IL-6 data, studies on selected samples indicated no substantial increase in the levels of IFN-γ or IL-1β during the time frame of this study (data not shown).

Nitrate/Nitrite Release

Nitrate and nitrite are oxidation products of NO. When the perfusate did not contain BSA (Control), the levels of nitrate/nitrite in the perfusate were close to zero after 1 or 2 h of perfusion. Additionally, the levels were low for the 3-h perfused Control group, resulting in a release rate of 1.81 nmol/g liver/min at 3 h. However, when the perfusate contained BSA (Albumin), the concentrations of nitrate/nitrite in the perfusate were relatively high, resulting in the release rates of 3.39 ± 2.82, 6.56 ± 3.97, and 7.45 ± 1.47 nmol/g liver/min at the end of perfusion for the 1-, 2-, and 3-h perfused Albumin groups, respectively. The release rates in the livers perfused for 3 h with BSA (Albumin) were significantly ($p < 0.005$, unpaired *t* test) higher than that for the 3-h Control livers.

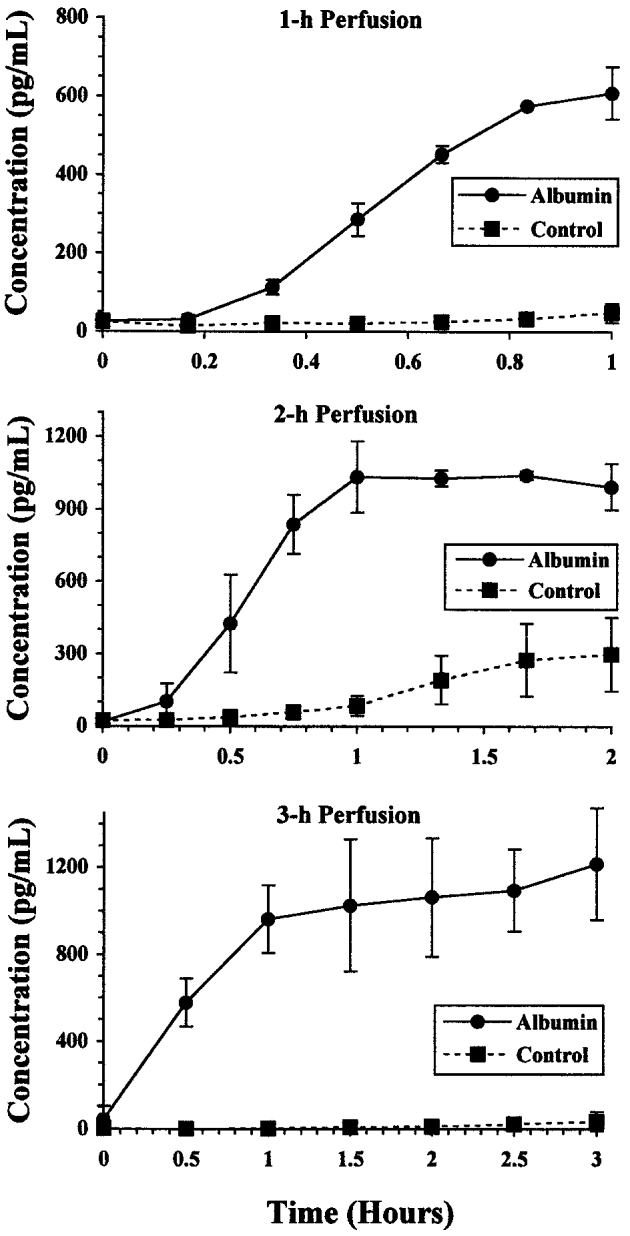

Fig. 1. The concentration–time courses of TNF-α in the outlet samples from isolated rat livers perfused with a perfusate containing 1% BSA (Albumin) or no BSA (Control) for 1, 2, or 3 h. *Symbols* and *bars* represent the average and SD values, respectively.

Bile Flow Rates, Transaminase Levels, and Liver Weights

The bile flow rates for different collection intervals are reported in Fig. 3 for the 3-h perfusion groups. Overall, the

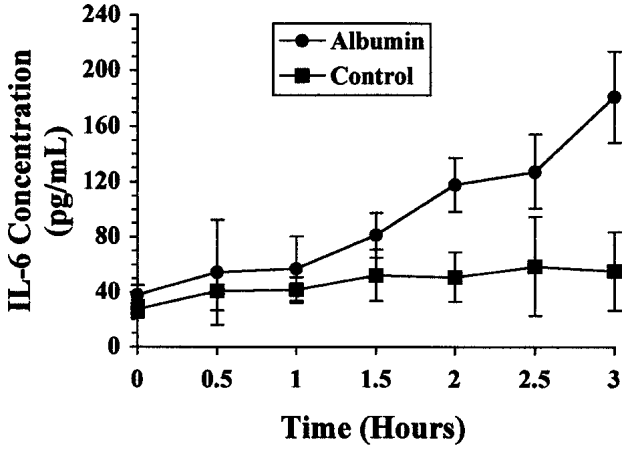

Fig. 2. The concentration–time course of IL-6 in the outlet samples from isolated rat livers perfused for 3 h with a perfusate containing 1% BSA (Albumin) or no BSA (Control). *Symbols* and *bars* represent the average and SD values, respectively.

bile flow rates of Albumin groups were significantly (ANOVA, $p < 0.0001$) lower than those of Control groups (Fig. 3). Additionally, the time of bile collection had a significant ($p < 0.01$) impact on the bile flow rates. However, the effect of time seemed to be mostly within the Albumin group, as the flow rates substantially decreased with time for livers perfused with BSA. On the other hand, the bile flow rates for the livers perfused in the absence of BSA (Control) remained relatively constant over the 3-h collection time (Fig. 3). The bile flow rates for the 1- and 2-h groups (data not shown) were similar to those observed for the 3-h group during the 0–1 and 0–2 h time intervals, respectively (Fig. 3).

The glutamic–oxaloacetic transaminase and glutamic–pyruvic transaminase levels were low during the entire period of perfusion with no significant difference between the perfusate groups. The mean (± SD) values of the wet liver weight (as a percentage of the body weight) at the end of liver perfusion were 3.52 ± 0.31, 3.07 ± 0.12, 3.74 ± 0.17, 3.54 ± 0.39, 3.56 ± 0.42, and 3.47 ± 0.4 for the 1-h Albumin, 1-h Control, 2-h Albumin, 2-h Control, 3-h Albumin, and 3-h Control, respectively, with all the values for individual livers being less than 4% of the body weight.

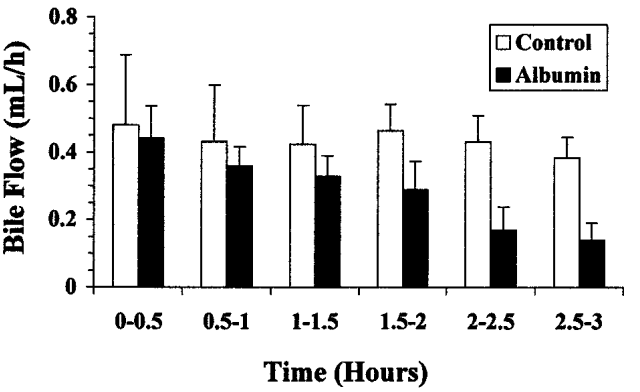

Fig. 3. Bile flow rates at different collection intervals in rat livers perfused for 3 h with a perfusate containing 1% BSA (Albumin) or no BSA (Control). The bile flow rates of Albumin groups were significantly lower than those of the control groups (ANOVA, $p < 0.0001$). *Columns* and *bars* represent the average and SD values, respectively.

Total CYP Levels and Cytochrome c Reductase Activities

The total CYP contents and cytochrome c reductase activities of microsomes prepared from different groups of livers are depicted in Fig. 4. For the 3-h perfusion group, livers perfused in the presence of BSA (Albumin) showed a 41% decrease ($p < 0.0001$) in total CYP levels when compared with the livers perfused with no BSA perfusate (Control) (Fig. 4, top). However, no significant differences were observed ($p > 0.05$) in CYP levels between Control and Albumin groups perfused for 1 or 2 h (Fig. 3, top).

Profiles obtained for cytochrome c reductase activities (Fig. 4, bottom) were similar to those obtained for total CYP levels (Fig. 4, top); compared with their respective Controls, livers perfused for 3 h with BSA (Albumin) showed a 31% decline ($p = 0.010$) in the levels of cytochrome c reductase activity. Additionally, similar to the CYP data, no significant differences were observed ($p > 0.05$) in cytochrome c reductase levels between Albumin and Control groups when the livers were perfused for 1 or 2 h (Fig. 4, bottom).

CYP Isoform Activities and Protein Levels

The effects of perfusate composition and duration of perfusion on ELISA-detectable protein levels and activities of CYP3A2, CYP2C11, and CYP2E1 are demonstrated in Figs. 5–7, respectively. Perfusion of livers for 3 h with the BSA-

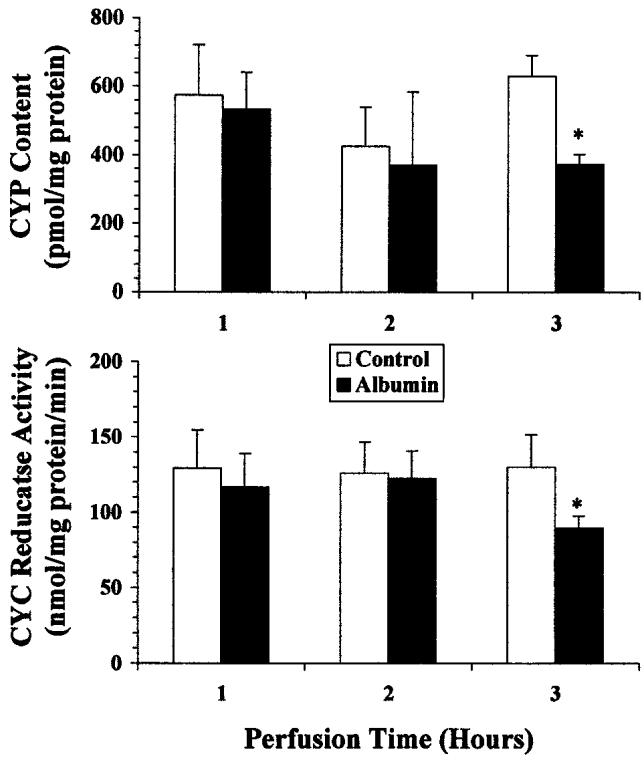

Fig. 4. Cytochrome P450 (CYP) contents (top) and cytochrome c reductase (CYC) activities (bottom) in rat livers perfused for 1, 2, or 3 h in the absence (Control) or presence (Albumin) of BSA in the perfusate. *Significant difference ($p < 0.05$) between Albumin and Control livers. *Columns* and *bars* represent the average and SD values, respectively.

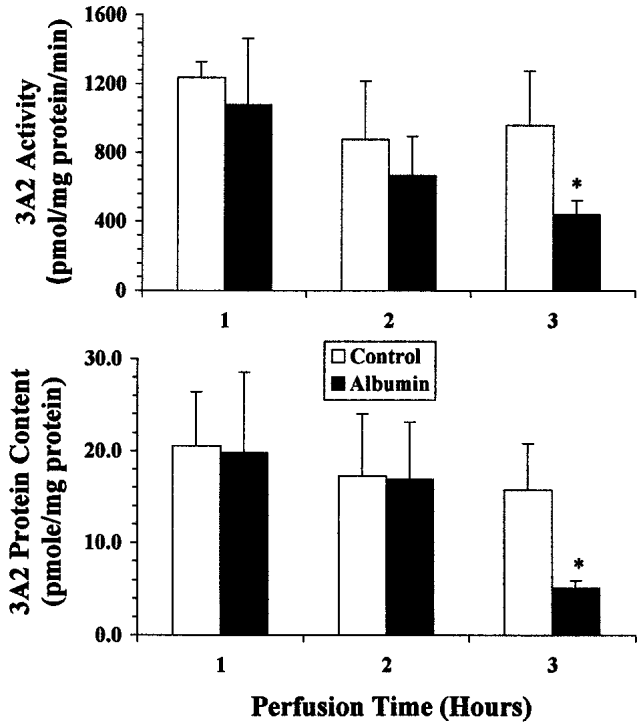

Fig. 5. The activities (top) and protein levels (bottom) of CYP3A2 in livers perfused for 1, 2, or 3 h in the absence (Control) or presence (Albumin) of BSA in the perfusate. The activities are based on testosterone 6β–hydroxylation. *Significant difference ($p < 0.05$, unpaired *t* test) between Albumin and Control livers. Additionally, there were significant differences ($p < 0.05$, ANOVA) between the 1- and 2-h and 1- and 3-h perfusion groups for the activity and between 1- and 3-h perfusion groups for the protein content. Columns and bars represent the average and SD values, respectively.

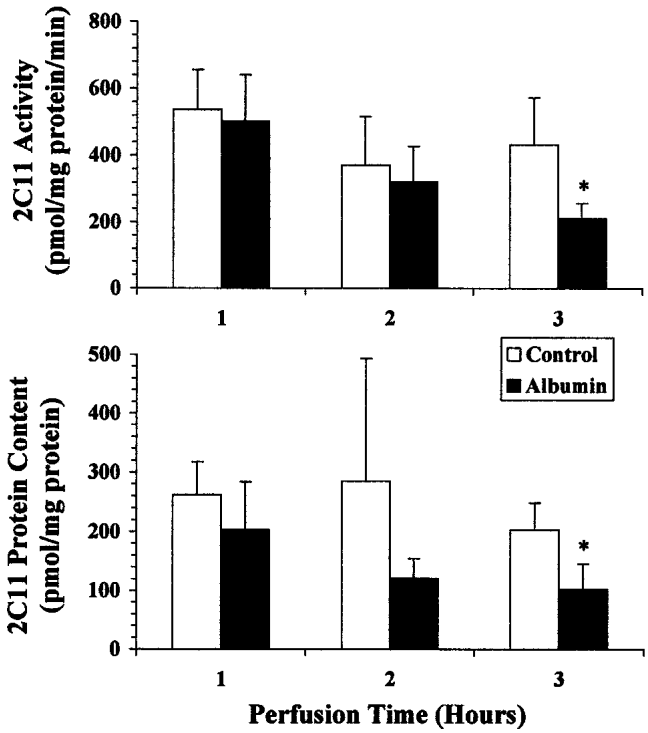

Fig. 6. The activities (top) and protein levels (bottom) of CYP2C11 in livers perfused for 1, 2, or 3 h in the absence (Control) or presence (Albumin) of BSA in the perfusate. The activities are based on testosterone 16α-hydroxylation. *Significant difference ($p < 0.05$, unpaired *t* test) between Albumin and Control livers. Additionally, there were significant differences ($p < 0.05$, ANOVA) between the 1- and 2-h and 1- and 3-h perfusion groups for the activity. *Columns* and *bars* represent the average and SD values, respectively.

containing perfusate significantly ($p < 0.02$) decreased both the activities and protein contents of these three CYP isoforms. However, BSA had no significant effect ($p > 0.05$) on either the activity or the protein contents of the isoforms in livers perfused for 1 or 2 h (Figs. 5–7). The changes in the activities of these isoforms as a result of BSA were similar to changes in their protein levels. The degrees of reduction in the activities and protein levels after perfusion of the livers for 3 h with Albumin were, respectively, 54% and 68% (CYP3A2, Fig. 5), 51% and 50% (CYP2C11, Fig. 6), and 66% and 59% (CYP2E1, Fig. 7).

In contrast to the testosterone and *p*-nitrophenol hydroxylations, dextromethorphan demethylation, which is an indication of CYP2D1 activity in rats, was not affected ($p > 0.05$) by the perfusate composition (Table I). Additionally, dextromethorphan demethylation remained relatively constant over the 3-h perfusion time in both Control and Albumin groups (Table I). Likewise, the dealkylation activities of both ethoxyresorufin and benzyloxyresorufin were similar ($p > 0.05$) in Albumin and Control livers (Table I). However, for ethoxyresorufin, a time-dependent decrease in the dealkylation activity was observed ($p < 0.0001$), regardless of the perfusion composition (Table I); compared with the data for 1-h perfusion, the activity decreased by 34% and 72% for Control livers and by 48% and 77% for Albumin livers after 2 and 3 h of perfusion, respectively (Table I). Additionally, although the benzyloxyresorufin dealkylation activity remained constant between 1 and 2 h of perfusion, a significant decrease ($p < 0.01$) was observed in the activities of the 3-h perfused livers when compared with the 1- or 2-h groups (Table I).

DISCUSSION

The isolated perfused rat liver (IPRL) model is a powerful *ex vivo* tool for delineating the metabolism of drugs in the intact organ while avoiding confounding factors that may be present *in vivo*. In this model, the liver is perfused *ex vivo* with a physiologic buffer often containing albumin. In addition to being an oncotic agent (6), preventing liver swelling during perfusion, albumin may have drastic effects on the kinetics of metabolism of drugs that are bound to this protein. For drugs bound to albumin, various models of hepatic elimination (19) predict a significant decrease in the hepatic extraction ratio and clearance for low-extraction-ratio drugs and a significant increase in the hepatic availability of high-extraction-ratio drugs in the presence of this protein in the perfusate. Therefore, it is not surprising to see that various investigators (7,8) have used albumin to delineate the effects of protein binding on the kinetics of hepatic metabolism and/or uptake of different drugs. However, during the development of an IPRL model to study the kinetics of release of cytokines, we recently (9) noticed that most commercial preparations of albumin, which have been used by us or other investigators in the past, contain LPS and would significantly stimulate the release of the proinflammatory cytokine,

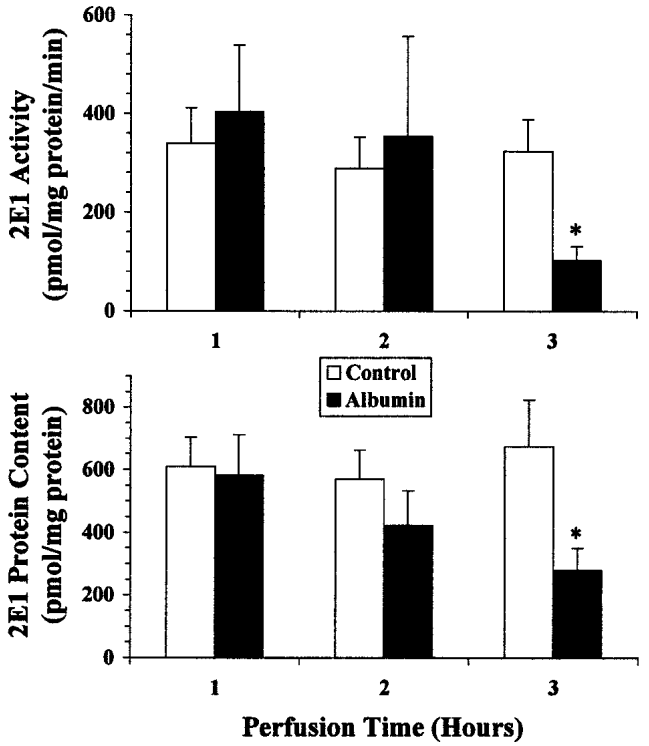

Fig. 7. The activities (top) and protein levels (bottom) of CYP2E1 in livers perfused for 1, 2, or 3 h in the absence (Control) or presence (Albumin) of BSA in the perfusate. The activities are based on *p*-nitrophenol hydroxylation. *, Significant difference ($p < 0.05$, unpaired *t* test) between Albumin and Control livers. *Columns* and *bars* represent the average and SD values, respectively.

TNF-α. Because proinflammatory cytokines are known to inhibit hepatic drug metabolism (reviewed in Ref. 5), we speculated that IPRLs perfused with an albumin-containing perfusate might cause a reduction in hepatic drug metabolism through cytokine stimulation. Indeed, the results presented here are in agreement with this postulate.

Because LPS is known to affect CYP drug metabolism (5), the BSA-induced reduction in CYP-mediated metabolism observed here is likely caused by the endotoxin content of BSA. The BSA preparation used in our studies contained 5 ng/mg endotoxin. For a flow rate of 30 mL/min, perfusion of livers for 1, 2, or 3 h with the perfusate containing 1% (w/v) BSA would result in an infusion of 90, 180, or 270 μg LPS, respectively, into the livers. Previous studies by others (20) and us (9) have shown that infusion of comparable amounts of LPS into IPRLs would result in substantial changes in the liver functions including an increase in the cytokine levels in the perfusate. In agreement with these reports (9,20), livers perfused with BSA showed substantially higher levels of TNF-α (Fig. 1) and to a lesser degree high levels of IL-6 (Fig. 2), compared with those perfused in the absence of BSA.

A majority of studies demonstrating an effect of inflammation- or infection-induced increases in the cytokine levels on drug metabolism have been conducted ≥12 h after the intervention (21,22). Therefore, an important question for our studies was whether the BSA-induced increases in the levels of TNF-α, observed in our previous studies (9), could induce substantial changes in drug metabolism during the relatively short course of liver perfusion studies. Although some IPRL studies may use longer perfusion times, most studies are concluded within 3 h of perfusion. Therefore, our drug metabolism studies were conducted after 1, 2, or 3 h of perfusion. Whereas BSA did not have any effect in livers perfused for 1 or 2 h, it resulted in significant decreases in total CYP content, the activities of cytochrome c reductase, and the activities and protein contents of CYP3A2, CYP2C11, and CYP2E1 after 3 h of perfusion (Figs. 4–7).

The relatively short time course of the effects of LPS-containing BSA on the CYP drug metabolism observed in our studies is not totally without precedent. For instance, Roe *et al.* (23) demonstrated that although the maximum effect of LPS injection on CYP2E1 activity in rats was observed after 48 h, significant reductions in the activity were measurable as early as 4 h after the LPS injection. The reduction in CYP2E1 activity followed an even earlier (1 h after LPS injection) reduction in nuclear protein binding of hepatocyte nuclear factor 1, a binding site responsible for up to 90% expression of CYP2E1 gene. Additionally, in agreement with our results (Fig. 7), the CYP2E1 activities at ≤ 2 h after the injection of LPS, were not significantly different from those of controls (23).

The pattern of BSA-induced changes in CYP-mediated drug metabolism observed in our IPRLs is qualitatively comparable with those observed in various *in vivo* and *in vitro* experimental models utilizing LPS (reviewed in Ref. 5). Sewer and Morgan (24) reported that the *in vivo* administration of endotoxin to rats decreased total CYP content and the activities and protein expression of CYP3A2, CYP2C11, and CYP2E1, findings in agreement with the data obtained in our IPRL model (Figs. 4–7). These authors (24) proposed a biphasic metabolic activity response to LPS. First, an increase in the production of NO would result in an early (< 6 h) reduction in the catalytic activity of some enzymes such as CYP2C11 and CYP3A2 in the absence of any changes in the gene expression or protein content of these enzymes. This effect was attributed to a reversible inhibition of the enzymes by NO because at later times, when the NO levels declined, this effect was no longer present. Second, LPS would cause a reduction in the gene expression and protein for CYP2C11 and CYP3A2, which appear to be prominent later (~24 h) after the LPS injection. This latter effect was demonstrated to be independent of NO production. Similarly, Takemura *et al.* (25) showed that within 8 h after the administration of LPS to rats, the CYP content declined significantly and remained low at 24 h. Whereas the administration of an inhibitor of NO synthase prevented the early (8 h) decrease in CYP content, it did not have any effect on the CYP reduction at 24 h, suggesting that only the early decrease in CYP content is NO dependent. Because metabolic activities were reduced in our experiments within a relatively short period of time (3 h) and were associated with an increase in NO levels in the Albumin perfusate, it is likely that NO may have played a role in our model.

The BSA-induced early changes in the metabolic activities in IPRLs were not indiscriminately applicable to all the CYP isoforms because no changes were observed in the activities of CYP1A (ethoxyresorufin dealkylation), CYP2B (benzyloxyresorufin dealkylation), or CYP2D1 (dextromethorphan demethylation) (Table I). Although Monshouwer *et al.* (3) reported a significant decline in the CYP1A and

Table I. Effects of Perfusate Containing 1% BSA (Albumin) or No BSA (Control) on the Activities (Mean ± SD) of Different CYP Isoforms Measured by Metabolism of Specific Substrates[a]

Perfusion time (h)	Group	Dextromethorphan demethylation (CYP2D1)[b]	Ethoxyresorufin dealkylation (CYP1A1)[c,*]	Benzyloxyresorufin dealkylation (CYP2B1/2)[c,**]
1 h	Control	87.5 ± 9.3	20.7 ± 7.4	9.05 ± 3.06
	Albumin	88.5 ± 9.0	25.8 ± 10.3	9.59 ± 2.52
2 h	Control	69.5 ± 18.6	13.7 ± 4.0	9.72 ± 3.59
	Albumin	62.7 ± 21.1	13.3 ± 1.1	9.58 ± 2.19
3 h	Control	76.0 ± 23.2	5.75 ± 0.98	5.49 ± 2.08
	Albumin	63.6 ± 4.0	5.97 ± 0.94	4.13 ± 1.31

[a] n = 4 for all groups except the 3-h Control group (n = 5).
[b] dpm/min/mg protein.
[c] pmol/min/mg protein.
* Significant differences ($P < 0.05$, ANOVA) in enzyme activities between the 1- and 2-h, 1- and 3-h, and 2- and 3-h perfusion groups.
** Significant differences ($P < 0.05$, ANOVA) in enzyme activities between the 1- and 3-h, and between 2- and 3-h perfusion groups.

CYP2B activities at 24 h after the *in vivo* injection of LPS in rats, we are not aware of any report investigating the early effects of LPS or other models of inflammation on the activities of these enzymes. Nevertheless, a substantial decline in the ethoxyresorufin and benzyloxyresorufin dealkylation with perfusion time in both Albumin and Control livers (Table I) suggest that IPRLs with long perfusion times should be used with caution for studies of the metabolism of CYP1A and CYP2B substrates.

The reductions in the metabolic activities of CYP isoforms observed in our study (Figs. 5–7) could arise from a change in the catalytic activity of the enzymes and/or a reduction in the enzyme protein levels. Recently, ELISA methods have been used for the estimation of protein concentrations of various CYP isoforms in microsomal preparations. However, it has been recognized (21) that the absolute values of these proteins should be viewed with caution because, in most cases, the sum of the isoform protein levels exceeds the total CYP protein content that is measured spectrophotometrically. Nevertheless, these estimates are useful, at a minimum, for comparative purposes when different treatments are applied. We selected CYP2E1, CYP3A2, and CYP2C11 for further protein measurements because of the significant reductions observed in their activities (Figs. 5–7) and also because the methods (18) and reagents for their analysis are available. Interestingly, the magnitude of the BSA-induced decreases in the activities of these isoforms after 3 h of perfusion were very similar to the degree of decline in their protein contents (Figs. 5–7). Similarly, lack of significant changes in the activities of these isoforms after 1 or 2 h of perfusion with BSA perfusate was consistent with the lack of significant alterations in the protein levels in these groups (Figs. 5–7). These data suggest that the BSA-induced reductions in the metabolic activities of CYP2E1, CYP3A2, and CYP2C11 after 3 h of perfusion are secondary to reductions in their respective protein levels.

It is generally believed that the suppressive effects of LPS on CYP metabolism are mostly mediated by major pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (5). However, in our model, the decrease in CYP metabolism was mainly accompanied by high levels of TNF-α achieved early (60 min) after the start of BSA perfusion (Fig. 1). Tumor necrosis factor-α has also been implicated by many others (2,26) in down-regulation of CYP drug metabolism. For example, Nadin *et al.* (26) have reported decreases in activities associated with CYP2C11 and CYP3A2, after 1 and 3 days of TNF-α treatment. The mechanism by which TNF-α down-regulates CYP-mediated drug metabolism may be via induction of nitric oxide synthase, resulting in an increase in NO levels (4). The latter has been directly implicated in the down-regulation of CYP metabolism (27). Additionally, TNF-α also stimulates the production and secretion of other cytokines including IL-1, IL-6, and IFN-γ, which have also been shown to down-regulate the CYP metabolism (5). However, in our studies, which are short term, only a relatively small increase in the levels of IL-6 was observed (Fig. 2) with no detectable levels of IL-1 or IFN-γ. Because IL-6 also results in suppression of CYP2C11 and CYP2E1 (28), a role for this cytokine in the inhibition of CYP cannot be ruled out. However, because of their time courses (Figs. 1 and 2), the role of TNF-α may be greater than that of IL-6 in our model.

The bile flow rates in Albumin livers were consistently lower than those in Control livers, with the differences increasing at later collection intervals (Fig. 3). This may be because our perfusate contained taurocholic acid for induction and maintenance of bile flow rates. Because albumin is known to bind to taurocholic acid, decreasing its hepatic uptake (29), the lower flow rates in Albumin livers (Fig. 3) are likely related to the lower availability of taurocholic acid in these livers. Additionally, LPS is known to adversely affect bile salt uptake and bile flow rate (30), perhaps contributing to the differences between the livers perfused with LPS-containing Albumin and Control perfusates (Fig. 3). Nevertheless, despite the dependence of bile flow rates on factors such as the perfusate composition, this parameter is useful for determination of viability within each group of livers subjected to the same experimental condition.

In conclusion, addition of BSA to perfusates, which is a routine practice in isolated rat liver studies, may reduce CYP-mediated drug metabolism in this model. The mechanism of this new observation is independent of the known BSA-induced reduction in metabolism though an increase in the protein-bound fraction of drugs. The BSA effect on drug metabolism occurs after 3 h of perfusion and is not apparent after 1 or 2 h of perfusion. Therefore, metabolism studies that use IPRL models with BSA-containing perfusates and duration of ≥ 3 h may need reevaluation. Further experiments are currently in progress to determine unequivocally the exact

mechanism(s) involved in the BSA-induced decreases in hepatic drug metabolism.

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