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Differential Effect of Pentoxifylline on Lipopolysaccharide-Induced Downregulation of Cytochrome P450

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ABSTRACT. It is now established that inflammatory stimuli such as lipopolysaccharides (LPS) and polyinosinic acid:polycytidylic (polyIC) suppress hepatic expression of cytochrome P450 (P450) genes in rat liver. Previous studies have suggested that LPS- or polyIC-induced downregulation of P450 was due to endogenously released inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-6, and interferons (IFNs). To improve our understanding of the role of inflammatory cytokines in mediating P450 depression, we investigated the possibility of preventing P450 downregulation with pentoxifylline. Pentoxifylline has been shown to inhibit LPS-induced TNF-α production by suppression of TNF-α gene expression. The present study shows that in uninduced male rats pentoxifylline selectively prevents the downregulation of microsomal P4501A2 and P4502B caused by LPS. No protective effect of pentoxifylline on the downregulation of P4502E1 and P4503A1/2 was observed. PolyIC-induced downregulation of P4501A2, P4502B, P4502E1, and P4503A1/2 was not affected by pentoxifylline. These results suggest that the LPS-induced downregulation of P4501A2 and P4502B is mediated to a large extent by TNF-α. Other cytokines might be involved in the suppression of P4502E1 and P4503A1/2. The fact that polyIC-induced downregulation is not protected by pentoxifylline is further evidence that this agent acts via a selective induction of IFNs. BIOCHEM PHARMACOL 52;8:1195–1200, 1996.

KEY WORDS. cytochrome P450; pentoxifylline; lipopolysaccharide; downregulation; polyIC, tumor necrosis factor

Inflammatory stimuli such as polyIC§ or LPS depress hepatic microsomal P450 [1–3]. Depression of P450 causes profound changes in the capacity of the liver to metabolize drugs, ultimately resulting in a decreased clearance of drugs [4, 5]. The polyIC-induced downregulation of P450 is mediated through interferon α/β [6], whereas in the suppressive effect of LPS, proinflammatory cytokines such as TNF-α, interleukin-1α (IL-1α), and interleukin-6 (IL-6) are involved [5, 7, 8].

TNF-α is thought to be a proximal mediator of the inflammatory response induced by LPS and most likely triggers the release of other cytokines. Administration of LPS or human recombinant TNF-α to mice or rats resulted in depression of P450, which was similar for both treatments [8, 9]. Therefore, regulation of TNF-α production and function is considered as a potential target in preventing the downregulation of P450 during inflammatory reactions.

Recently, the phosphodiesterase inhibitor pentoxifylline, a methylxanthine derivative (1-(5′-oxohexyl)-3,7-dimethylxanthine) that appears to be beneficial in the clinical treatment of vascular disorders, has raised new interest by inhibiting LPS-induced TNF-α production *in vivo* and *in vitro* through the suppression of TNF-α gene transcription [10, 11]. Furthermore, pentoxifylline has been shown to increase animal survival in lethal models of infection [12] and sepsis [13].

These findings suggest that pentoxifylline may be capable of preventing the LPS-mediated downregulation of P450 by reducing TNF-α levels. To investigate this possibility and to improve our understanding of the role of cytokines in P450 downregulation, we evaluated the *in vivo* effects of pentoxifylline on the downregulation of P450 induced by either LPS or polyIC in rats.

MATERIALS AND METHODS

Materials

All reagents and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA), except as noted below.

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§ *Abbreviations:* P450, cytochrome P450; LPS, lipopolysaccharide; polyIC, polyinosinic acid:polycytidylic; TNF-α, tumor necrosis factor α; IFN, interferon.

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Animals and Treatment

Male Sprague-Dawley rats (175–200 g) obtained from Charles River Labs (Montreal, Quebec, Canada) were housed on clay chips and allowed free access to Purina rat chow and water *ad libitum*. Rats were allowed to acclimatize in our facilities for 5 days before use. The animals were treated with LPS plus saline (n = 6), LPS plus pentoxifylline (n = 6), polyIC plus saline (n = 4), polyIC plus pentoxifylline (n = 4), saline plus pentoxifylline (n = 4), or saline plus saline (n = 6). *Escherichia coli* LPS (O127:B8) and polyIC were dissolved in pyrogen free saline and injected i.p. at a dose of 0.5 mg/kg and 10 mg/kg, respectively. Pentoxifylline was dissolved in pyrogen free saline and injected i.p. 30 min before and 30 min after LPS or polyIC treatment at a dose of 100 mg/kg per injection. Equal volumes of saline were injected when no LPS, polyIC, or pentoxifylline was administered. All animals were killed 24 hr after LPS or polyIC treatment.

Microsomal Drug Biotransformation

Hepatic microsomes were prepared by differential centrifugation as described [14] and were resuspended in 0.1 M phosphate buffer (pH 7.5) containing 25% glycerol. Microsomes were stored until use at −70°C. Microsomal protein was measured by the method of Lowrey *et al.* [15], and total cytochrome P450 content was determined according to Omura and Sato [16]. Ethoxyresorufin O-deethylase and pentoxyresorufin O-dependentylase activities were determined by the method of Burke *et al.* [17]. Para-nitrophenol hydroxylase and erythromycin N-demethylase activities were determined as described by Koop [18] and Arlotto *et al.* [19], respectively.

Western Blot Immunoassay

Microsomal proteins were loaded on a 7.5% sodium dodecylsulfate (SDS)-polyacrylamide gel and separated by electrophoresis under nonreducing conditions [20]. After the proteins were transferred to nitrocellulose membranes by a semi-dry transfer process (Electrophoretic Transfer System, Tyler Research Instruments, Edmonton, Canada), immunodetection was performed according to standard protocols [21]. P4501A2 and P4502E1 were detected with polyclonal rabbit antirat P4501A1 and P4502E1 (OXYgene Dallas, Dallas, TX, USA) and were visualized with an alkaline phosphatase-labeled secondary antibody with nitro blue tetrazolium and 5-bromo-4-chloro-indolyl phosphate as substrates. In this study, we used a P4501A1 antibody that cross reacts with P4501A2. In uninduced male Sprague-Dawley rats, P4501A1 is a minute fraction of the total P450 amount and as a consequence only P4501A2 is detected.

Microsomal proteins per lane were loaded as indicated in the figure captions.

Slot Blot Analysis of RNA

P4501A2 mRNA was quantitated by slot blot analysis of total mRNA with a mouse P4501A1 cDNA probe. This probe, obtained from the American Type Culture Collection (Rockville, MD, USA), detects both P4501A1 and P4501A2 [3]. Total RNA was isolated from 200 mg of liver as described previously [22]; 30 μg of total RNA was denatured by heating at 60°C for 15 min in the presence of 7% formaldehyde and loaded onto a nitrocellulose membrane with a slot blot apparatus (Bio-Rad Bio-Dot TM SF Apparatus, Biorad, Richmond, CA, USA) [23]. The RNA was loaded in columns in twofold serial dilutions in 20× SSC (3 M NaCl, 300 mM sodium citrate) starting with 10 μg RNA. The membranes were air dried and the RNA immobilized by baking for 2 hr at 80°C. The cDNA P4501A1 probe was hybridized under the following conditions. Prehybridization and hybridization were carried out as described by Christou *et al.* [24]. Briefly, membranes were prehybridized overnight at 42°C in 5× SSPE, 5× Denhardt's [25], 50% formamide, 0.1% SDS, and 200 μg/mL salmon sperm DNA. Hybridization was carried out in the same buffer containing 10% dextran sulfate and α-^{32}P-labeled P4501A1 cDNA. The membrane was washed twice for 15 min in 2× SSPE plus 0.1% SDS at room temperature, followed by two 15-min washes in 0.1× SSPE plus 0.1% SDS at 60°C. The membranes were then stripped and total mRNA quantitated with the polyT oligonucleotide as described by Harley [26].

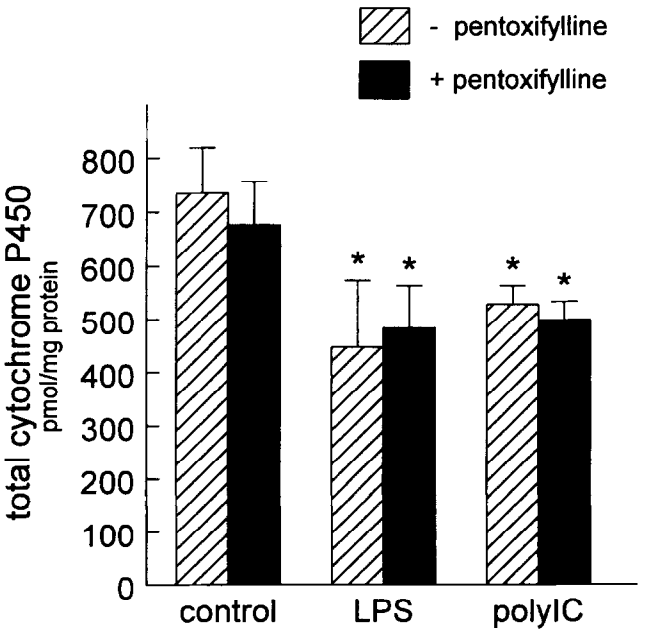

FIG. 1. Effect of pentoxifylline on the LPS- (0.5 mg/kg) or polyIC- (10 mg/kg) induced downregulation of total P450 in hepatic rat microsomes. Pentoxifylline (100 mg/kg) was administered 30 min before and 30 min after LPS or polyIC treatment. Rats were killed 24 hr after LPS or polyIC treatment, and hepatic microsomes were prepared. Values are means ± SD for 4–6 rats per group. Statistical analysis was done by Student's *t*-test. Significantly different from control, *$P < 0.05$.

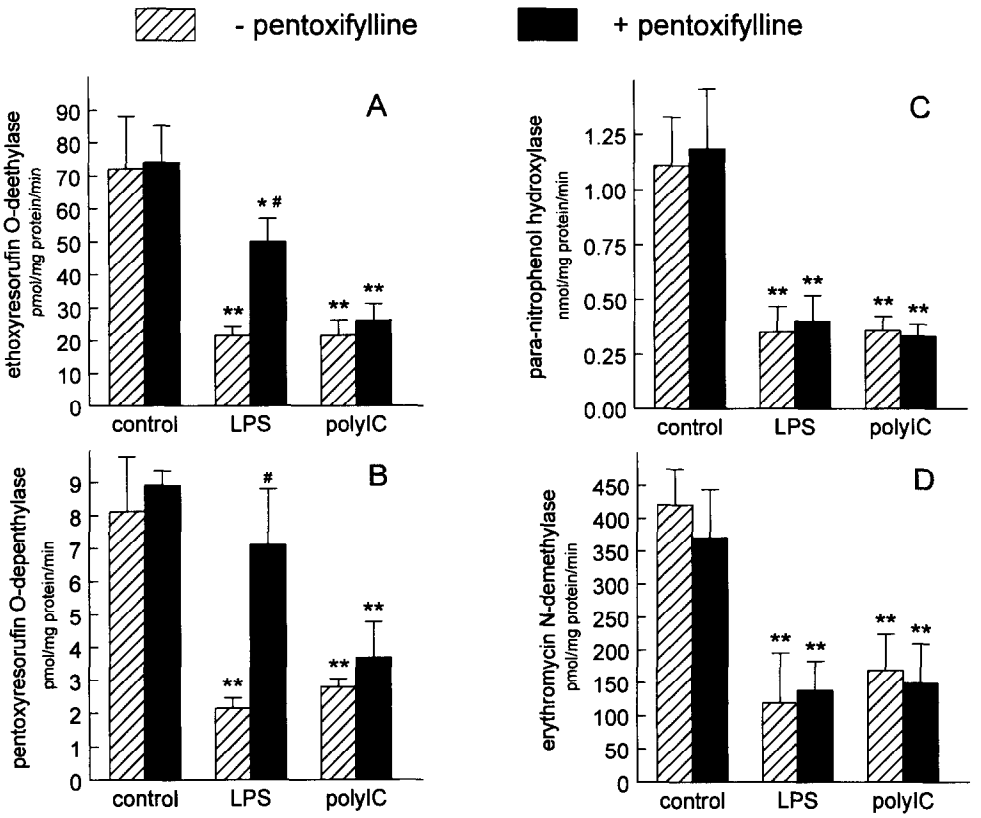

FIG. 2. Effect of pentoxifylline on the LPS- (0.5 mg/kg) or polyIC (10 mg/kg)- induced downregulation of four P450-dependent monooxygenase activities in hepatic rat microsomes. Pentoxifylline (100 mg/kg) was administered 30 min before and 30 min after LPS or polyIC treatment. Rats were killed 24 hr after LPS or polyIC treatment, and hepatic microsomes were prepared. Values are means ± SD for 4–6 rats per group. Statistical analysis was done by Student's *t*-test. Significantly different from control, *$P < 0.05$, **$P < 0.01$. Significantly different from LPS without pentoxifylline, #$P < 0.05$. A: Ethoxyresorufin O-deethylase. B: Pentoxyresorufin O-depenthylase. C: Para-nitrophenol hydroxylase. D: Erythromycin N-demethylase.

Analysis of Results

All the data are presented as means ± SD. Student's *t*-test for unpaired data was used for comparison of the differences between two means. *P* values of less than 0.05 were accepted as evidence of a statistically significant difference.

RESULTS

The administration of a single dose of the interferon inducer polyIC (10 mg/kg) or LPS (0.5 mg/kg) to uninduced male rats resulted in a significant depression of total P450 content after 24 hr (Fig. 1). Both polyIC and LPS depressed total P450 to the same extent. Administration of pentoxifylline 30 min prior to and 30 min after polyIC, LPS, or saline treatment had no effect on total P450 levels (Fig. 1).

The effect of polyIC or LPS on four P450-dependent monooxygenase activities is shown in Fig. 2. All enzyme activities (ethoxyresorufin O-deethylase, pentoxyresorufin O-depentylase, para-nitrophenol hydroxylase, and erythromycin N-demethylase) appeared significantly lower in polyIC- or LPS-treated rats. PolyIC and LPS were almost equally effective in the depression of enzyme activities, ranging from minimal 60% to maximal 73%. Pentoxifylline was ineffective in preventing the polyIC-induced depression of all enzyme activities determined (Fig. 2A–D). Furthermore, the LPS-induced downregulation of para-nitrophenol hydroxylase and erythromycin N-demethylase activities were also unaffected by pentoxifylline (Fig. 2C,D). However, pentoxifylline partially prevented the depression of ethoxyresorufin O-deethylase and pentoxyresorufin O-depentylase activities caused by LPS. After LPS treatment in combination with pentoxifylline, ethoxyresorufin O-deethylase activity was decreased by only 30% (Fig. 2A). The protective effect of pentoxifylline on pentoxyresorufin O-depentylase activity was even more pronounced (Fig. 2B), resulting in no difference between the control and LPS-treated groups (8.1 ± 1.7 vs. 7.1 ± 1.7 pmol/mg protein/min). In addition, the pentoxyresorufin O-depentylase activity of the control group treated with pentoxifylline, which was slightly higher than that of the

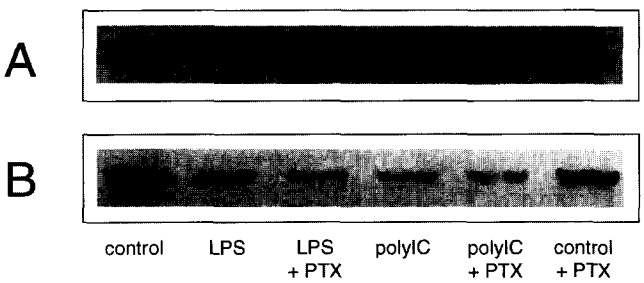

FIG. 3. Representative Western blots of P4501A (A) and P4502E1 (B) from rat liver microsomes. Western blot was performed as described in Materials and Methods. Protein loading was 40 μg (A) or 20 μg (B) of microsomal protein per lane. PTX, pentoxifylline.

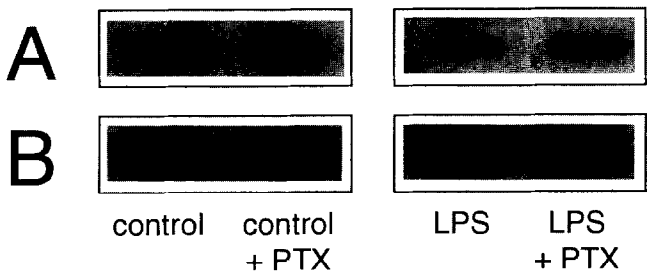

FIG. 4. Representative slot blot analysis of total RNA extracted from rat liver. Slot blot was obtained by probing the same blot with the P4501A1 cDNA probe (A) and the polyT oligonucleotide (B). PTX, pentoxifylline.

control group, was not different from the LPS in combination with the pentoxifylline group.

Western blot analysis demonstrated that the decrease in ethoxyresorufin O-deethylase and para-nitrophenol hydroxylase activities was associated with lower levels of P4501A2 and P4502E1 apoprotein (Fig. 3A,B). Furthermore, pentoxifylline only prevented the decrease in P4501A2 apoprotein levels by LPS. No effect of pentoxifylline was observed on P4502E1 apoprotein downregulation by either LPS or polyIC.

The protective effect of pentoxifylline on P4501A2 apoprotein downregulation by LPS was accompanied by a diminished loss of P4501A2 mRNA levels (Fig. 4).

DISCUSSION

Many studies have been conducted on the depression of P450-mediated hepatic drug metabolism during inflammatory reactions induced by either LPS or polyIC. It was shown that this depression was associated with a loss of mRNA and subsequent P450 apoprotein synthesis in the liver [27–29].

The effects of LPS and polyIC on four constitutive P450 activities and apoprotein levels presented here are in good agreement with previous studies [1, 3, 9]. It is remarkable that in the present study LPS (0.5 mg/kg) and polyIC (10 mg/kg) were almost equally effective in decreasing P450 enzyme activities. The depression of P450 is rather nonselective as the expression of all constitutive P450 isoenzymes determined are downregulated after LPS or polyIC treatment.

Previous studies have shown that treatment of animals with inflammatory cytokines such as TNF-α, IL-1, IL-6, or IFNs caused a downregulation of P450. The decrease in P450 activity during infection may be due to these endogenously released cytokines. However, the sensitivity of various constitutive P450 isoenzyme activities toward cytokines was variable [9, 30–32].

In human hepatocytes in culture, it has been shown that TNF-α is the most potent depressor of P4501A [33, 34]. Other proinflammatory cytokines such as IL-1, IL-6, and IFN-γ were also able to suppress P450 enzymes [33, 35].

Several studies have demonstrated that serum levels of TNF-α dramatically increased within 2 hr after LPS administration [36]. No significant increase in TNF-α has been observed after polyIC treatment. The methylxanthine derivative pentoxifylline, at a dose of 100 mg/kg, has been shown to prevent almost completely the LPS-induced increase in serum TNF-α levels [13, 37].

In the present study, the polyIC-induced downregulation of P450 was not prevented by a similar dose of pentoxifylline. This result is not surprising because IFNs could be the primary cytokines involved in P450 downregulation by polyIC. However, the LPS-induced decrease in ethoxyresorufin O-deethylase and pentoxyresorufin O-dependentylase activities, corresponding to P4501A and P4502B, respectively, was partially prevented by pentoxifylline. No effect was observed on para-nitrophenol hydroxylase and erythromycin N-demethylase activities, corresponding to P4502E1 and P4503A1/2, respectively. These results suggest that TNF-α is the major cytokine involved in the LPS-induced downregulation of P4501A2 and P4502B but is very likely not the only cytokine responsible for the downregulation of P4502E1 and P4503A1/2. The idea that cytokines have differential effects on P450 downregulation is supported by previous findings. Chen *et al.* [9] demonstrated in uninduced male rats that ethoxyresorufin O-deethylase and pentoxyresorufin O-dependentylase activities were hardly affected by IL-6, whereas both TNF-α and IL-1 decreased all P450 enzyme activities determined. However, in rat hepatocytes, P4502B was more sensitive to IL-1β or IL-6 than to P4501A1 [32]. Given that LPS induces several inflammatory cytokines and that pentoxifylline decreases serum TNF-α levels without significantly affecting other cytokine levels, the downregulation of P4502E1 and P4503A1/2 may be mediated by other cytokines, whereas P4501A2 and P4502B are mainly downregulated by TNF-α.

Berthou *et al.* [38] described another methylxanthine derivative, caffeine, that increased P4501A- and P4502B-dependent monooxygenase activities in rats pretreated for 3 days with 150 mg/kg/day of caffeine. It is unlikely that the protective effect of pentoxifylline in this study is due to induction because pentoxifylline itself had no effect on any of the P450-dependent enzyme activities determined.

In conclusion, this study shows that pentoxifylline, an inhibitor of TNF-α production, selectively prevents microsomal P4501A2 and P4502B downregulation caused by treatment of uninduced male rats with LPS, suggesting that this downregulation is TNF-α dependent. The role of TNF-α in LPS-induced downregulation of P4502E1 and P4503A1/2 is not predominant, as pentoxifylline showed no protective effect. The fact that P450 downregulation induced by polyIC is not protected by pentoxifylline is further evidence that polyIC acts via a selective induction of IFNs.

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