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Lipopolysaccharide opposes the induction of CYP26A1 and CYP26B1 gene expression by retinoic acid in the rat liver in vivo

Reza Zolfaghari, **Christopher J. Cifelli**, **Siam O. Lieu**, **Qiuyan Chen**, **Nan-qian Li**, and **A. Catharine Ross**
Department of Nutritional Sciences, Pennsylvania State University, University Park, Pennsylvania

Abstract

Retinoic acid (RA), a principal metabolite of vitamin A (retinol), is an essential endogenous regulator of gene transcription and an important therapeutic agent. The catabolism of RA must be well regulated to maintain physiological concentrations of RA. The cytochrome *P*450 (CYP) gene family CYP26, which encodes RA-4-hydroxylase activity, is strongly implicated in the oxidation of RA. Inflammation alters the expression of numerous genes; however, whether inflammation affects CYP26 expression is not well understood. We investigated the regulation of CYP26A1 and CYP26B1 mRNA levels by RA and LPS in the rat liver, as the liver is centrally involved in retinoid metabolism and the acute-phase response to LPS. Both CYP26A1 and CYP26B1 mRNA were induced in <4 h by a single oral dose of all-*trans*-RA. RA-induced responses of both CYP26A1 and CYP26B1 were significantly attenuated in rats with LPS-induced inflammation whether LPS was given concurrently with RA or after the RA-induced increase in CYP26A1 and CYP26B1 mRNA levels. When RA and LPS were administered simultaneously (6-h study), LPS alone had little effect on either CYP26A1 or CP26B1 mRNA, but LPS reduced by 80% the RA-induced increase in CYP26A1 mRNA ($P < 0.02$), with a similar trend for CYP26B1 mRNA. When LPS was administered 4 h after RA (16-h study), it abrogated the induction of CYP26A1 ($P < 0.02$) and CYP26B1 ($P < 0.01$). Overall, these results suggest that inflammation can potentially disrupt the balance of RA metabolism and maintenance of RA homeostasis, which may possibly affect the expression of other RA-regulated genes.

Keywords

cytochrome *P*-450; transcriptional regulation; poly-I:C

Retinoic acid (RA), the principal bioactive metabolite of dietary vitamin A (retinol), is an essential regulator of gene expression and an important factor in the regulation of cell proliferation and differentiation (5, 41). Due to their differentiation-promoting properties, retinoids have become important drugs in the treatment of a variety of skin disorders, leukemias, and other cancers (4). All-*trans*-RA functions as the principal endogenous ligand of nuclear hormone receptors of the RA receptor (RAR) family (RAR-α, RAR-β, and RAR-γ), which combine with proteins of the retinoid X receptor (RXR) family to form heterodimeric transcription factor complexes that bind to RA response elements (RAREs) in the regulatory region of genes capable of responding directly to RA (5). In vivo, RA is rapidly taken up from plasma into the liver and other tissues and turns over quickly (2, 3, 16). Thus, the physiological control of RA-responsive genes is likely to depend on both

Address for reprint requests and other correspondence: A. C. Ross, Pennsylvania State Univ., S-126 Henderson Bldg. South, Univ. Park, PA 16802, acr6@psu.edu.

well-regulated production of RA from vitamin A and on the capacity for RA oxidation when its level is elevated.

The liver plays a central role in retinoid oxidation and the production and excretion of polar metabolites (28, 32, 40). Although the oxidation of RA was first ascribed to proteins of the cytochrome *P*-450 (CYP) family many years ago (37), only more recently have specific genes encoding RA oxidizing activity been identified and cloned and their expressed proteins studied for their enzymatic activity. The CYP gene family designated CYP26 (34) encodes enzymes with RA-4-hydroxylase activity that are capable of converting RA into polar metabolites, including 4-hydroxy- and 4-oxo-RA, which subsequently can be further metabolized to aqueous-soluble compounds, such as retinoyl-β-glucuronide, a compound found in bile (20, 53). In mammals, three evolutionarily conserved genes, CYP26A1, CYP26B1, and CYP26C1, have been shown to display different yet partially overlapping expression patterns during embryonic development and in adult tissues (17, 33, 34, 43, 50, 51). Functionally, all-*trans*-RA is the preferred substrate of CYP26A1 and CYP26B1, whereas CYP26C1, which appears to be expressed at low levels in adult tissues, metabolized *cis*- and all-*trans* isomers of RA (17, 29, 34, 43, 44, 51). A distinguishing feature of the CYP26A1 gene is the presence in its 5′-regulatory region of two or more RAREs capable of binding RAR-RXR complexes (26, 27). Generally, CYP26A1 mRNA is expressed at low or undetectable levels until the concentration of RA becomes elevated. CYP26A1 mRNA was strongly induced in the liver of rats fed a diet high in vitamin A and was increased dose dependently after acute administration of all-*trans*-RA (49). CYP26B1, which shares ~43% amino acid sequence identity with CYP26A1 (29), has been studied mostly during development and in neural tissues (1, 29, 47), but this gene is also regulated by RA in adult parenchymal tissue (11, 46). However, the manner by which RA regulated CYP26B1 expression has not been established. The differences in tissue distribution and substrate selectivity among these CYP26 genes suggest that each may serve a somewhat different function with respect to the regulation of retinoid homeostasis.

Inflammation is known to alter the expression and activity of numerous genes, including several genes of the CYP superfamily (for a review, see Ref. 31). In physiological studies, retinoids have generally shown anti-inflammatory activity (22). However, the relationship between inflammation and RA metabolism is not well understood. In previous studies that we conducted in the human monocytic cell line THP-1, we observed that, whereas CYP26A1 mRNA was increased by RA, as anticipated, the induction by RA was significantly reduced in cells that were also treated with LPS (12). LPS is a principal ligand of Toll-like receptor (TLR)4 and one of the most common models for studying inflammation (8, 25, 48). Previously, we used a model of low-dose LPS administration to determine the impact of acute inflammation on retinol transport (38). In the present investigation, we used the same model, together with RA administration, to examine the regulation of CYP26A1 and CYP26B1 mRNA by RA and LPS in the liver, as this organ is centrally involved in both RA metabolism and the response to LPS. The results of these experiments show that LPS opposes the induction of CYP26A1 and CYP26B1 mRNA when RA and LPS are administered simultaneously. Moreover, LPS can abrogate the RA-induced increase in CYP26A1 and CYP26B1 mRNA after the initiation of the inductive response. These data suggest that both the positive regulation of the CYP26A1 and CYP26B1 genes by retinoids and their negative regulation by inflammatory stimuli contribute to the balance of RA metabolism and the maintenance of retinoid homeostasis.

MATERIALS AND METHODS

Animals and experimental designs

Protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University. Female Sprague-Dawley rats were either purchased from Charles River Laboratories (Boston, MA) or obtained by breeding in our animal facility under conditions that have been described previously (15).

To evaluate the effects of RA and LPS on gene expression, three independent experiments were conducted in which RA, LPS, and RA in combination with LPS were administered on different schedules, as denoted in the figures. Briefly, rats fed a purified vitamin A-adequate diet (4 mg retinol/kg diet, Research Diets, New Brunswick, NJ) (36) were treated with an oral dose of all-*trans*-RA (500 μg, resembling a high therapeutic dose) in ~30 μl of vegetable oil (49) or an equal amount of oil as a placebo for RA. A single intraperitoneal injection of 50 μg/100 g body wt of *Pseudomonas aeruginosa* LPS (List Biological Laboratory, Campbell, CA) was administered in sterile PBS (38) with an equal volume of PBS as the placebo control. Three rats were treated with *Escherichia coli* LPS (type 055:B5) in place of *P. aeruginosa* LPS. As the results did not differ with the type of LPS, these groups were combined for statistical analysis. Food was removed after the administration of LPS (38). The duration of the three experiments differed, as RA and LPS were either coadministered 6 h before, or RA was administered 16 h before and LPS 12 h before, the collection of tissues. In one experiment, a subset of rats (n = 3–4 rats/group) of control, RA, or RA + LPS-treated rats was lightly anesthetized by isoflurane-oxygen inhalation and then received 0.15 ml/100 g body wt of albumin-bound [^{3}H]RA. The preparation of the dose and collection of tissues, 90 min after the dose, were similar to methods described in Ref. 13. Plasma total retinol and liver total retinol were determined after saponification and extraction of retinol using a reverse-phase HLPC method with detection at 325 nm and trimethylmethoxyphenyl retinol as an internal standard for quantification (39).

RNA isolation and detection of CYP26A1 mRNA by Northern blot analysis

Total RNA was prepared from individual rat livers and then pooled within treatment groups, and poly(A)$^{+}$-enriched RNA was prepared (55). Four micrograms of poly(A)$^{+}$ RNA was subjected to electrophoresis in agarose gel and then transferred to a Nytran membrane by gravity (49). Prehybridized membranes were hybridized with full-length CYP26A1 cDNA labeled with [α-^{32}P]dCTP, washed, and exposed to Kodak BioMax X-ray film (Eastman Kodak, Rochester, NY) (49).

Nuclei isolation

Fresh liver tissue (4 g) was homogenized in 40 ml of cold 0.3 M sucrose and 10% glycerol-containing *buffer A* [10 mM Tris·HCl (pH 7.5), 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 0.5 mM PMSF, and 1 mM DTT] in a glass Dounce homogenizer with 20 strokes. Each homogenate was passed through two layers of gauze and centrifuged at 1,650 *g* for 15 min at 2°C. The cytosol was retained, and the pellet was suspended in 10 ml of cold 1.6 M sucrose-containing *buffer A* and then layered onto 10 ml of cold 1.6 M sucrose-containing *buffer A*. Tubes were centrifuged at 8,000 *g* for 30 min as described above. The pellet was resuspended in 3 ml of storage buffer containing 40% glycerol, quantified, and rapidly frozen in a dry ice-ethanol bath for storage at −80°C (56).

Nuclear runon assay

An aliquot of nuclei (0.5–1 mg DNA) was used to run the assay in the present of 200 μCi of [α-^{32}P]UTP (Amersham, Chicago, IL) under conditions similar to those described previously (56). The labeled RNA was extracted with Trizol-LS (Invitrogen), purified on a

G-25 RNA spin column (Roche, Indianapolis, IN) to remove any unincorporated nucleotides, and then hybridized to Nytran membranes dot blotted with linearized DNA. The membrane was washed and then exposed to X-ray film (Kodak BioMax, Eastman Kodak) for times of 1 h to 3 days at −80°C.

Quantitative real-time PCR of CYP26A1 and CYP26B1 mRNA in livers of RA- and LPS-treated rats

Total RNA (1 μg) from individual rat livers was first reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Promega). The diluted reaction product (1/20) was used for real-time PCR analysis using 2× Real Time SYBR Green/Fluorescein PCR Master Mix (SuperArray Bioscience, Frederick, MD) in a final volume of 25 μl. The PCR cycling program was first set at 95°C for 10 min to activate the *Taq* polymerase and then at 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s using the DNA Engine2 Opticon with Continuous Florescence Detector (MJ Research, Watertown, MA). The primers used for PCR were 5′-GTGCCAGTGATTGCTGAAGA-3′ (sense) and 5′-GGAG-GTGTCCTCTGGATGAA-3′ (antisense) for rat CYP26A1 (Genbank Accession No. DQ266888), 5′-TTGAGGGCTTGGAGTTGGT-3′ (sense) and 5′-AACGTTGCCATACTTCTCGC-3′ (antisense) for rat CYP26B1 (Genbank Accession No. NM_181087), and 5′-CGCGGT-TCTATTTTGTTGGT-3′ (sense) and 5′-AGTCGGCATCGTTTAT-GGTC-3′ (antisense) for rat 18S rRNA (Genbank Accession No. X01117). Each RNA transcript was measured separately and calculated using 18S rRNA as an internal control. Data were normalized to the average value for the control group, set at 1.00, before statistical analysis. Alternatively, CYP26A1 was quantified by PCR in which [α-^{33}P]dATP was incorporated into the nucleotide master mix, as described previously (12). Labeled amplicons were separated by electrophoresis through a polyacrylamide gel, after which the gel was dried and exposed to X-ray film for visualization, and the amplicon bands were then cut out and counted by liquid scintillation spectrophotometry for final quantification.

Statistics

Results were analyzed by ANOVA using SuperAnova software (Abacus, Berkeley, CA), followed by a least-squares means test to determine significant differences ($P < 0.05$) among treatment groups. Linear regression analysis was also performed using Super-Anova software.

RESULTS

LPS-induced inflammation opposes the early response of CYP26A1 and CYP26B1 to RA

In previous studies, we established a model of low-dose LPS administration, using *P. aeruginosa* LPS at 50 μg/100 g body wt, to create a state of mild inflammation (38). In the present study, we employed the same LPS treatment in conjunction with RA administration to examine the effect of inflammation on CYP26A1 and CYP26B1 expression. In preliminary studies, we observed a very rapid increase in the expression of CYP26A1 and CYP26B1 mRNA in the liver of RA-treated rats, with maximum effects by 6–10 h, and therefore we first conducted a study of 6-h duration to determine if LPS opposes the induction of CYP26A1 and CYP26B1 expression by RA during the initial rise in CYP26A1 gene expression. By Northern blot analysis (Fig. 1*A*), the relative abundance of CYP26A1 mRNA in control rats [shown in *lanes 1*, *5*, and *9* for 3 separate pools of liver poly(A)$^+$ RNA] was low, whereas, by comparison, it was highly induced in rats treated with RA alone for 6 h (*lanes 2*, *6*, and *10*). At this time, one or more larger-sized bands, which may represent nascent, partially processed RNA, were also detectable. The abundance of CYP26A1 mRNA in the liver of rats treated for 6 h with LPS alone (*lanes 3* and *7*) was

similar to that for the control group. However, the increase in expression due to RA was significantly attenuated by LPS in rats that received both RA + LPS at the same time (*lanes 4* and *8*).

To confirm that the increase of CYP26A1 mRNA in retinoid-treated rat livers was due to increased gene transcription, a nuclear run-on study was conducted (Fig. 1*B*). Nuclei were prepared from the liver of vitamin A-deficient rats before and 3 h after treatment with 120 μg Am580, a stable analog of RA used to maximize the detection of transcription. No signal was detected after 3 days of exposure for nuclei from the vitamin A-deficient liver, whereas a signal with significant intensity was observed (detectable in <1 day of exposure) in nuclei from the retinoid-treated liver. By comparison, signals with nearly equal intensity were observed for the reference genes β-actin and GAPDH in nuclei from vitamin A-deficient and Am580-treated rats. These results suggest that most, if not all, of the increase in CYP26A1 mRNA in the liver of rats treated with retinoids is due to an increase in the rate of transcription of the CYP26A1 gene.

Next, we tested whether the larger-sized transcripts of CYP26A1 mRNA observed in the liver of RA-treated rats are likely to represent primary message transcripts still in the nucleus and not yet fully processed. The liver homogenate from rats treated Am580, as above, was separated into three fractions (a whole homogenate, a nuclear fraction, and a cytosolic fraction) and subjected to Northern blot analysis (Fig. 1*C*). The larger-sized transcripts visible in *lanes 1* and *2* were apparent in the nuclear fraction (*lane 3*), whereas the major transcript seen in Fig. 1*A* and in *lanes 1* and *2* of Fig. 1*C* was present in both the nuclear fraction and cytoplasmic fraction (*lane 4*). Notably, however, the cytoplasmic fraction was nearly devoid of the larger-sized transcripts. Thus, the results of both nuclear run-on and subcellular separation experiments provided additional support that both gene transcription and mRNA processing were still ongoing in the period of time in which CYP26A1 mRNA expression was downregulated by LPS.

To quantify the results for CYP26A1, total liver RNA from individual rats was subjected to quantitative real-time PCR analysis (Fig. 1*D*). Rats treated for 6 h with RA showed an increase of ~25-fold in CYP26A1 mRNA, consistent with the Northern blot analysis. LPS alone did not alter CYP26A1 mRNA expression. However, LPS strongly attenuated the response to RA, by >80% in this study.

The expression of CYP26B1 was analyzed for the same samples (Fig. 2*A*). The magnitude of the response to RA alone was somewhat lower for CYP26B1 than for CYP26A1, similar to the previous microarray study. LPS alone did not significantly alter the level of expression of CYP26B1 mRNA, and the combination of treatment with RA and LPS resulted in a response intermediate to both the RA-treated and LPS-treated groups.

To determine the response of CYP26B1 to a range of doses of RA, liver samples from a previous study (49) of CYP26A1 expression were analyzed for CYP26B1 mRNA. The results showed a linear response over a wide range of RA dosages, indicating both sensitive and robust regulation of CYP26B1 expression by RA in the rat liver (Fig. 2*B*).

To further compare the response of CYP26A1 and CYP26B1 to these various treatments, linear regression was performed (Fig. 2*C*). The correlation between the responses of the two CYP26 genes was highly significant ($R = 0.76$, $P < 0.0001$).

LPS-induced inflammation abrogates the response of CYP26A1 and CYP26B1 to RA during the period of induction

As LPS strongly opposed the induction of CYP26A1 and CYP26B1 mRNA by RA when both LPS and RA were administered at the same time, we tested further whether LPS could abrogate the response to RA after gene expression was induced. In two experiments, vitamin A-adequate rats were first treated with a high dose of RA (500 μg/rat po) given 4 h before LPS was administered. CYP26A1 and CYP26B1 mRNA levels were then quantified 12 h after treatment with LPS. CYP26A1 expression was increased 4 h after RA (not shown, as similar to responses at 6 h in Fig. 1*A*) at the time that LPS was administered. We did not include a group of rats treated only with LPS alone since the results shown Fig. 1 had not shown any substantial effect of LPS alone. Sixteen hours after RA and 12 h after LPS, plasma retinol was reduced by ~15% by RA alone and by one-half by RA and LPS combined (Fig. 3*A*). These results confirmed that LPS had induced a mild state of hyporetinolemia, one of the markers of the acute-phase response (38).

CYP26A1 (Fig. 3*B*) and CYP26B1 (Fig. 3*C*) mRNA were each strongly increased in RA-treated rats. The prolonged response of CYP26A1 and CYP26B1 to RA in this 16-h study is likely due to the higher dose of RA used in this experiment (500 μg compared with 100 μg in the study shown in Fig. 1) and to the oral route of administration, which is likely to have prolonged the uptake of RA into the liver. When rats treated with RA were treated 4 h later with LPS, the increase in CYP26A1 mRNA was nearly completely attenuated. Similarly, the response of CYP26B1 mRNA to RA was nearly completely attenuated when treatment with RA was followed 4 h later by treatment with LPS.

A subset of the rats in this study received a single test dose of [^{3}H]RA administered intraperitoneally 90 min before the end of the experiment. The formation of aqueous-soluble metabolites, an end product of retinoid oxidation, was measured (Fig. 3*D*). ^{3}H-labeled aqueous-phase metabolites in the liver were significantly higher in RA-treated rats (Fig. 3). By comparison, ^{3}H-labeled aqueous-phase metabolites were lower in the liver of rats treated with RA + LPS compared with RA alone ($P = 0.061$) and similar to the control group. For all tissues combined (liver, small intestine, lung, and kidney, shown in Fig. 3), ^{3}H-labeled aqueous-phase metabolites were twice for liver alone, but differences due to treatment were similar to the liver. Thus, when CYP26A1 and CYP26B1 mRNA were first increased by RA, LPS could still attenuate the level of aqueous-phase metabolites in the liver and extra-hepatic tissues.

Poly-I:C, a ligand for TLR3, also abrogates the RA-induced CYP26A1 response

To determine if the abrogation of induction of CYP26A1 is specific for LPS or may apply to other types of inflammatory stimuli, we compared LPS and poly-I:C, an analog of viral double-stranded RNA that is known to rapidly induce the production of interferons and other cytokines, including TNF-α (35, 45) through stimulation of TLR3/mda-5 (18). CYP26A1 mRNA was barely detectable in the control group (*lane 1*), while the response of the RA-treated group (16 h) was very strong, as anticipated (Fig. 4*A*, *lane 3*). LPS alone (12 h) had little if any effect on CYP26A1 mRNA (*lane 4*), whereas RA + LPS dramatically reduced the RA-induced expression of CYP26A1 (*lane 2*). Poly-I:C alone (12 h, *lane 5*) did not alter CYP26A1 mRNA compared with the control level, whereas RA + poly-I:C, where poly-I:C was given 4 h after RA (*lane 6*), attenuated the increase due to RA. The quality and intensity of the RNA loading controls (Fig. 4*B*) were similar for all treatment groups. These results were confirmed for individual liver samples using [^{33}P]dATP incorporation during primer-specific PCR amplification of the CYP26A1 transcript to quantify the levels of CYP26A1 mRNA. Whereas RA alone increased CYP26A1 mRNA by more than fivefold, both LPS and poly-I:C reproducibly abrogated the response of CYP26A1 mRNA to RA (Fig. 4*C*).

DISCUSSION

RA is both an important endogenous regulator of gene expression and an important therapeutic drug. CYP26A1 has been hypothesized to function as a sensor-effector mechanism that is intimately involved in the control of tissue RA concentrations. The structure of the CYP26A1 gene, with two or more RAREs in the promoter region, is apparently poised for immediate upregulation in response to increased RA concentrations (26, 27). The rapid, high-level response of CYP26A1 may be especially significant in the liver, which has been shown to obtain the majority of its RA by uptake from plasma (23). The liver is perfused by portal blood from the intestine, in which RA is produced from vitamin A or carotenoid precursors during absorption and by blood returning from the peripheral tissues, many of which express several enzymes capable of producing RA from retinol. Previously, we showed that CYP26A1 mRNA in the liver of adult rats and mice is regulated by the vitamin A status under steady-state conditions and by exogenous all-*trans*-RA under conditions of acute administration (49, 52). Concomitantly, the metabolism of [^{3}H]RA by liver microsomal membranes was altered (14, 52). Little is known, however, of the mechanisms that may reduce the level of expression of CYP26A1 or attenuate its induction by RA. Moreover, the regulation of the CYP26B1 gene, while apparently capable of responding to RA in vivo (11, 46), is not well understood, and thus far no functional RARE have yet been reported. Recently, Balmer and Blomhoff (6) classified over 500 genes reported to be regulated by RA into categories of genes that are either directly regulated through classical RAR-RXR-mediated transcriptional activation or are indirectly regulated by RA through nonconventional pathways. Regarding the latter categories, some genes are physiologically responsive to RA but nevertheless lack an apparent RARE in proximity to the promoter region; some may be regulated by other factors in a secondary manner; and some may be regulated as the result of ligand-dependent interactions between nuclear retinoid receptors and other transcription factors, such as activator protein-1 (6).

Inflammation has become recognized as an important factor in the development of cardioavascular disease, diabetes, and other chronic diseases (7). LPS, a component of the cell wall of various gram-negative bacteria, has frequently been used to induce inflammation and investigate its consequences (for a review, see Ref. 9). The binding of LPS to its receptor, TLR4, which is present on the surface of numerous other types of cells (9, 21), triggers an intracellular signaling cascade that leads to multiple changes in gene expression (21). At least some of the effects of LPS have been attributed to the activation of the NF-κB and IL-6-related families of transcription factors, which are known to be regulatory factors for numerous hepatic genes. In previous studies using the same model of LPS-induced inflammation, using 50 μg *P. aeruginosa* LPS/100 g body wt as we have used in the present study, we reported that inflammation reduces the production and concentration of retinol-binding protein (RBP) in the rat liver as well as the plasma concentration of RBP and its ligand, retinol (38). The acute-phase response also results in reduced albumin (10), the carrier for plasma RA (42). Another study (13) has demonstrated alterations by LPS in the organ distribution and hepatic metabolism of RA. We initially hypothesized that LPS might downregulate the expression of CYP26A1 in the liver, in which CYP26A1 mRNA is most abundant, based in part on studies we had conducted in THP-1 cells, a human monocytic cell line that is induced to differentiate into macrophage-like cells by RA (12), and also on reports that other CYP superfamily genes expressed in the liver are significantly reduced in states of inflammation (31). However, we found that LPS alone had little effect on CYP26A1 mRNA in the rat liver, whereas CYP26B1 mRNA was increased slightly. Nevertheless, a strong suppressive effect of LPS on both CYP26A1 and CYP26B1 mRNA was apparent when RA was administered and gene expression was induced. Interestingly, the suppression of CYP26A1 and CYP26B1 mRNA levels by LPS was similar whether RA was administered before or concomitantly with LPS and was similar in female rats, as

shown, and in male rats (data not shown), indicating that this response is not gender specific. The opposition to RA-induced CYP26A1 expression by LPS was also similar between *P. aeruginosa* LPS and *E. coli* LPS, and, additionally, was also observed when the dose of *P. aeruginosa* LPS was reduced to 10 μg/100 g body wt (data not shown). Different types of purified LPS have been shown to elicit different patterns of cytokine and chemokine production (30), but we did not observe a difference between these two sources of LPS on the suppression of RA-induced CYP26A1 expression. Indeed, this suppression did not require LPS specifically, as poly-I:C, a synthetic double-stranded RNA that mimics viral RNA and is known as a potent inducer of type I and type II interferons (19, 24, 54), also suppressed the induction of CYP26A1 mRNA by RA. In human peripheral blood leucocytes and dendritic cells, poly-I:C also induced the expression of numerous other cytokines, including TNF-α (35). Thus, poly-I:C at the dosage we used may also induce a mild inflammatory response, and TNF-α could potentially be a common mediator of the responses to both LPS and poly-I:C.

In the present study, both LPS and poly-I:C significantly reduced CYP26 mRNA levels even after gene expression was induced by RA, suggesting that these agents could be affecting CYP26 mRNA levels by multiple mechanisms. In our present investigation, the transcriptional induction observed by nuclear run-on assay was associated with the accumulation of higher-molecular-weight mRNAs. It will be interesting to investigate in future studies by what molecular mechanisms LPS and poly-I:C, or their downstream mediators like TNF-α, can potently counteract the induction of CYP26A1 and CYP26B1 expression by RA. It will also be important to clarify the mediators of the action of LPS and poly-I:C on hepatic CYP26A1 and CYP26B1 expression and whether CYP26A1 and CYP26B1 expression is also regulated by LPS and poly-I:C in nonhepatic tissues.

In summary, the present study has demonstrated that the upregulation of CYP26A1 and CYP26B1 by RA in the liver is strongly opposed or abrogated by acute LPS-induced inflammation. The counteracting effect of LPS on RA-induced expression of CYP26A1 and CYP26B1 could potentially alter the balance of RA within cells, and thus could have consequences on the expression of other RA-responsive genes.

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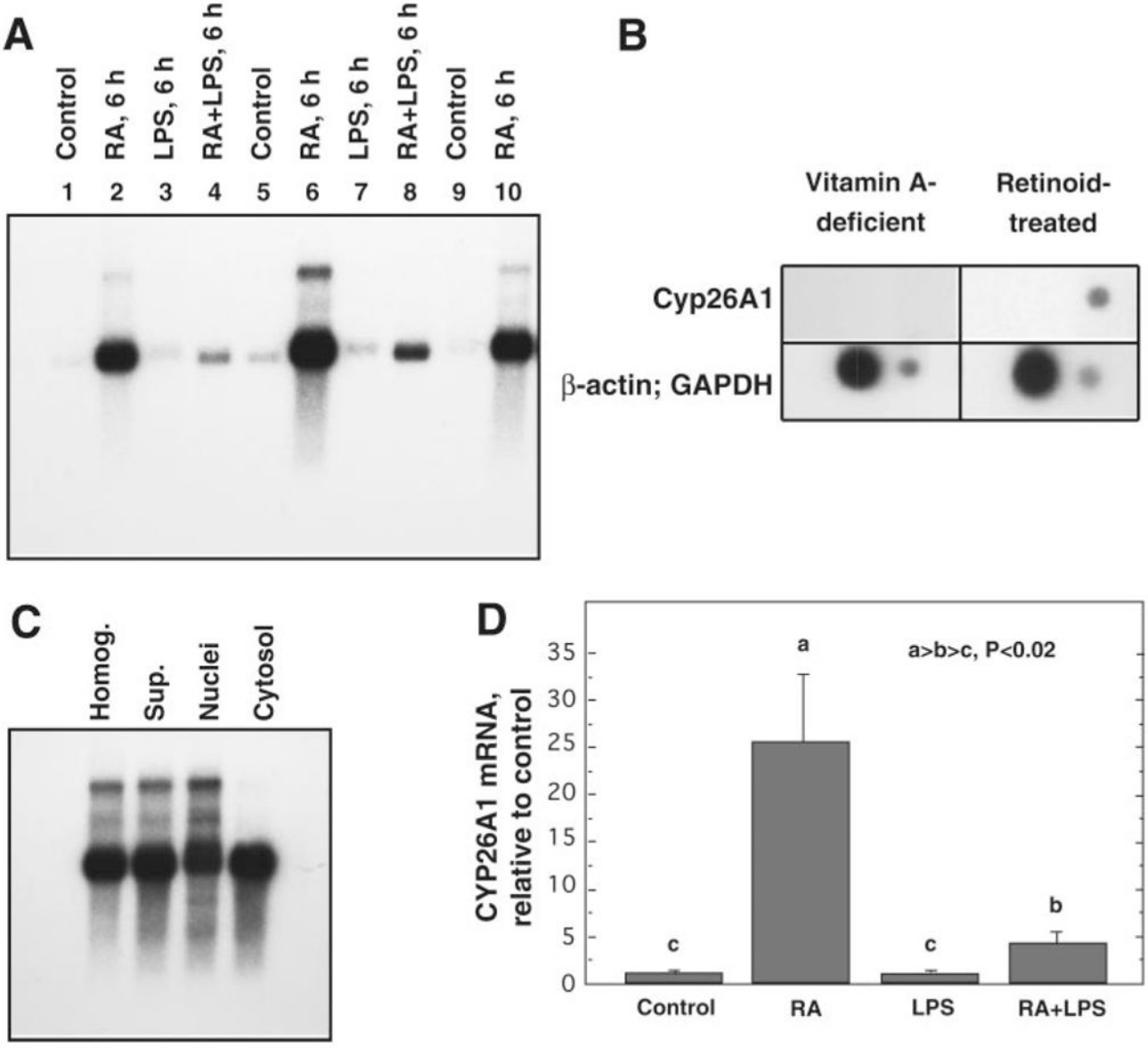

Fig. 1.

Cytochrome *P*-450 (CYP)26A1 expression in the liver of rats treated simultaneously with retinoic acid (RA) and LPS. *A*: Northern blot analysis of CYP26A1 mRNA 6 h after treatment with RA, LPS, and the combination of RA with LPS. Each lane contains poly(A)$^+$ RNA pooled equally from 3 rat livers. *B*: nuclear run-on assay of CYP26A1 gene transcription in the rat liver. Nuclei were isolated from pooled livers of vitamin A-depleted rats and rats treated with an RA analog, Am580, for 3 h (see MATERIALS AND METHODS) and used to synthesize nascent RNA transcripts in the presence of [^{32}P]UTP. Labeled RNA was extracted and hybridized to empty plasmid vector DNA (*top left*) or to a plasmid vector containing cDNA of CYP26A1 (*top right*). β-Actin (*bottom left*) and GAPDH (*bottom right*) were blotted onto the Nytran membrane. After a wash, membranes were exposed to X-ray film for 1.5 days for CYP26A1 and 20 h for reference genes. Signals for β-actin and GAPDH were essentially equal for both treatment conditions. *C*: liver fractionation and Northern blot analysis of CYP26A1 mRNA in rats treated as in *B*. RNA

was prepared from the whole homogenate (Homog), homogenate after filtration (Sup), washed nuclear pellet (Nuclei), and cytosolic fractions (Cytosol) and analyzed as in *A. D*: quantitative PCR analysis of CYP26A1 transcripts measured in the liver of individual rats (n = 6 rats/group) 6 h after simultaneous treatment with RA (500 μg orally), LPS (50 μg/100 g body wt ip), or the combination of RA + LPS as described in MATERIALS AND METHODS. For comparison, data were normalized to the expression of 18S rRNA and the mean value for the control group for CYP26A1 was set to 1.00, after which the other groups were compared with this value. Bars show means ± SE; statistical results (by a least-squares means test) are displayed.

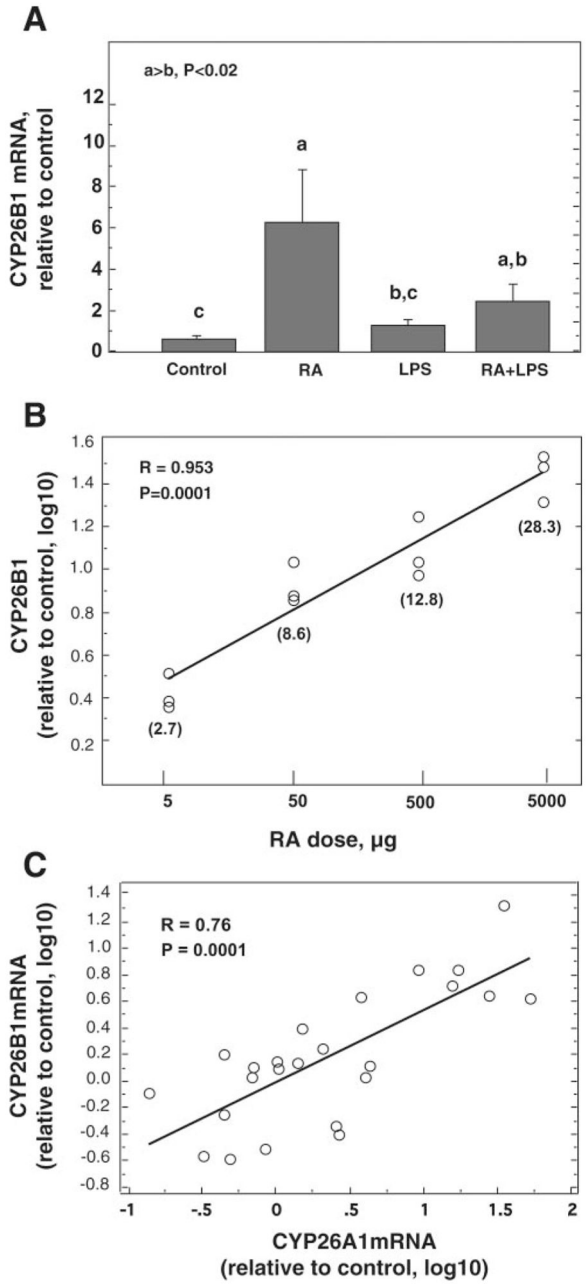

Fig. 2.

CYP26B1 expression in the liver of rats treated simultaneously with RA and LPS. *A*: quantitative PCR analysis of CYP26B1 transcripts measured in the liver of individual rats (*n* = 6 rats/group) 6 h after simultaneous treatment with RA (500 µg orally), LPS (50 µg/100 g body wt ip), or the combination of RA + LPS as described in MATERIALS AND METHODS. Data were normalized and referenced to the control group for CYP26A1, set to 1.00 as in Fig. 1*D*, to compare the relative expression values of these two genes with each other. Bars show means ± SE; statistical results (by a least-squares means test) are displayed. *B*: dose-response curves showing linear increases in CYP26B1 mRNA with increasing doses of RA (liver RNA samples are from rats in Ref. 49). *C*: linear regression

analysis of $\log^{10}$-transformed values for CYP26B1 mRNA in *A* and $\log^{10}$-transformed values for CYP26A1 mRNA from Fig. 1*D*.

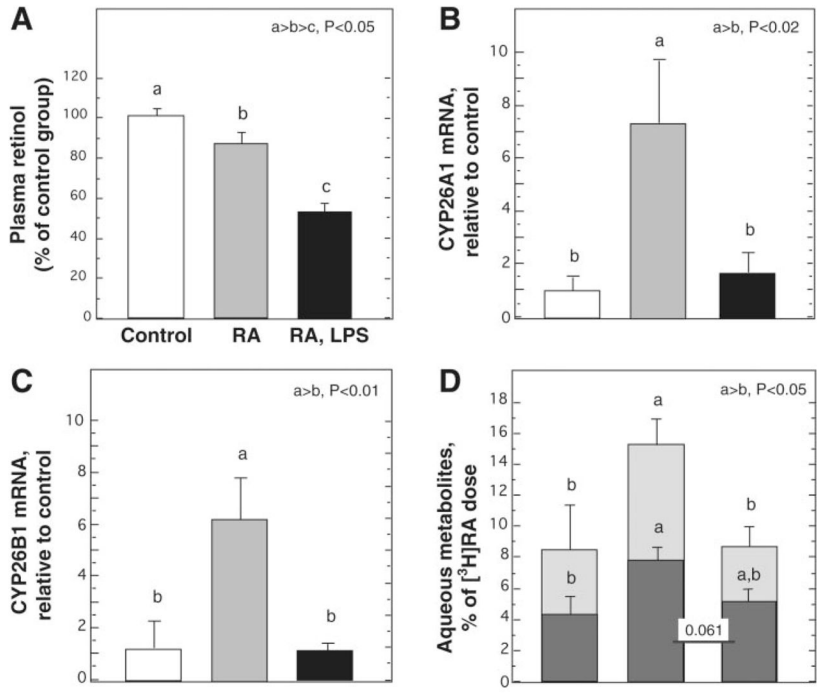

Fig. 3.

Plasma retinol, CYP26A1 and CYP26B1 transcripts, and retinoid metabolites in rats treated with LPS 4 h after the administration of RA. Rats were given 500 μg RA and then, 4 h later, LPS, and plasma and liver samples were collected 12 h later. *A*: changes in plasma retinol concentrations due to RA and RA + LPS. *B*: quantitative PCR analysis of CYP26A1. *C*: quantitative PCR analysis of CYP26A1 transcripts. Data were normalized to the control group and then expressed as means ± SE. *D*: aqueous-phase metabolites of RA formed after an intravenous administration of a test dose of [^{3}H]RA given 90 min before the end of the experiment to a subset of rats (n = 3–4 rats/group) in the same experiment. Bars (means ± SE) show data for the liver (dark gray bars) and for the lungs, small intestine, spleen, kidney, and liver combined (total bars). The value of 0.061 represents a borderline significant difference between RA- and RA + LPS-treated rats for aqueous-phase metabolites in the liver.

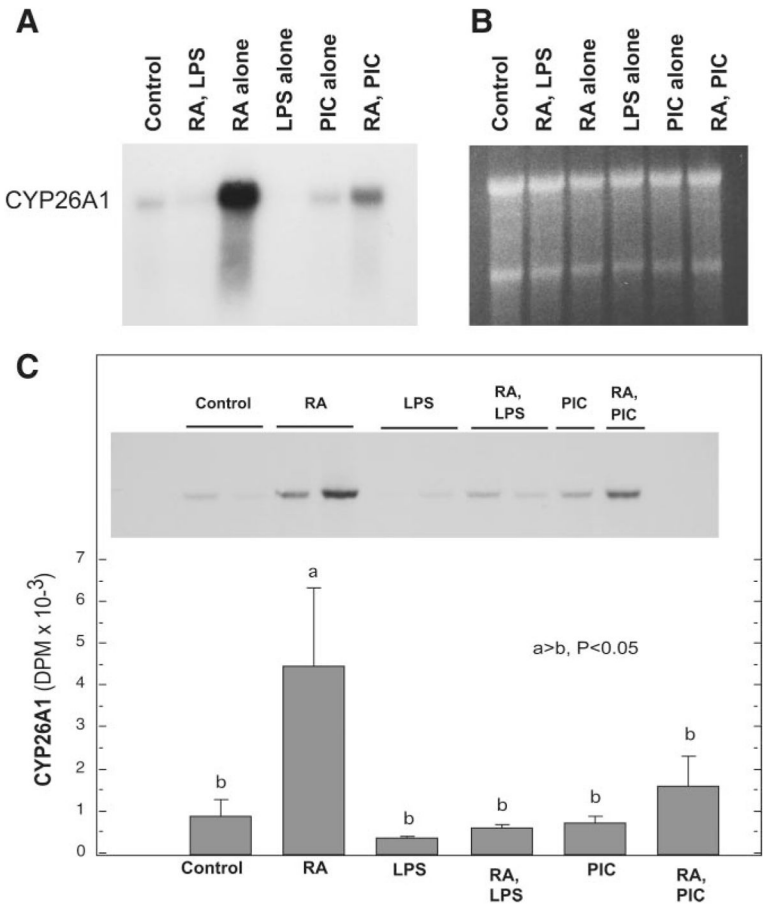

Fig. 4.

Poly-I:C (PIC), as well as LPS, abrogates the response of CYP26A1 after its induction by RA. Rats were treated with 500 μg RA or vehicle and then with LPS or PIC or vehicle 4 h later. *A*: CYP26A1 mRNA was detected by Northern blot analysis using poly(A)$^+$-enriched RNA from pooled rat livers from each treatment group. *B*: loading control, showing the ethidium bromide-stained agarose gel prior to transfer to the Nytran membrane for Northern blot analysis. *C*: RT-PCR am-plification of rat liver RNA using [^{33}P]dATP incorporation during PCR (see MATERIALS AND METHODS). RNA from n = 3–4 individual rats/group was subjected to PCR analysis. Bars show means ± SE. The *inset* shows results from 1 or 2 rats/group visualized by X-ray film exposure of the polyacrylamide gel before the gel was cut and counted.