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Role of Nitric Oxide in the Inhibition of Cytochrome P450 in the Liver of Mice Infected with *Chlamydia trachomatis*

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ABSTRACT. In this study, we attempted to determine the effect of a systemic infection with *Chlamydia trachomatis* on cytochrome P450(CYP)-dependent metabolism in mice. Furthermore, we wanted to assess if these effects were mediated through NO. BALB/c(H-2d) female mice were inoculated intraperitoneally with the C. *trachomatis* mouse pneumonitis (MoPn) biovar, and induction of NO synthase (NOS) was detected by measuring [NO_x] levels and inducible NOS protein content in peritoneal macrophages by Western blotting. Recovery of C. *trachomatis* from liver, lung, and spleen peaked at 4 days postinfection. Following cotreatment with N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, there was a significant increase in the intensity and the length of the infection. Six days after inoculation with C. *trachomatis*, CYP1A- and CYP2B-mediated metabolism in the liver of the mice was diminished up to 49% of control levels. However, when animals were treated with N^G-nitro-L-arginine methyl ester at days 4 and 6 postinfection, the decrease in the metabolism of CYP1A and CYP2B was largely blocked. These results suggest that C. *trachomatis* infection can depress cytochrome P450 in a manner similar to other types of infections and that NO is likely to be a mediator of this depression. This finding may be of significance to patients taking drugs that are metabolized by phase I enzymes during infections with some bacteria such as C. *trachomatis*. BIOCHEM PHARMACOL **55**;11: 1835–1842, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. *Chlamydia trachomatis*; cytochrome P450; nitric oxide

Over the last decade, large amounts of data demonstrating the involvement of NO in a variety of physiological and pathophysiological processes have emerged. Besides its role as an endothelium-derived relaxing factor and neurotransmitter, NO has been implicated in the killing of tumor cells and parasites [1]. In the last few years, the list of organisms shown to be affected by NO includes bacteria, viruses, protozoa, fungi, and helminths [2].

Chlamydiae are Gram-negative obligate intracellular bacteria that produce systemic and local infections in humans as well as in animals [3]. Recently, it was shown that T cell-mediated immunity against *Chlamydiae* is mediated, at least in part, by T cell-derived IFN-γ† induction of NOS in infected epithelial cells [4]. Furthermore, NO has been implicated as a mediator of nonspecific immune defense against *Chlamydia trachomatis in vitro* [4, 5] and *in vivo* [6]. The mechanisms by which NO can inhibit bacterial growth remain unknown. However, some of the major molecular targets of NO identified thus far are heme- and nonheme-iron, and iron-sulfur-containing proteins and thiols [7].

CYP is a major hemoprotein in the liver, which metabolizes a variety of xenobiotic and endogenous substances [8]. The P450 microsomal monooxygenase system is of crucial importance in chemotherapy since drug efficacy is related to biotransformation and/or elimination of the compound. It has been demonstrated repeatedly that activation of the host-defense mechanisms during an immune response results in inhibition of P450-mediated drug biotransformation in humans and in animals. The list of immunomodulators that have been reported to alter P450-mediated metabolism includes several bacteria such as *Corynebaterium parvum, Toxoplasma gondii, Listeria monocytogenes, Fasciola hepatica, Trypanosoma brucei, Mycobacterium butyricum*, and various types of *Schistosoma* [9]. However, no data are available on the effects of the members of the *Chlamydia* genus on CYP. Recently, we and others have implicated NO as a mediator of rat hepatic CYP down-regulation by LPS, a component of the wall of Gram-negative bacteria [10–12]. Therefore, we were interested in determining whether an infection with C. *trachomatis* would result in down-regulation of P450-dependent metabolism, and in assessing the mechanism involved in this process. Here, we report that the effects of an infection with C.

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† *Abbreviations:* 7-BROD, 7-benzyloxyresorufin O-debenzylation; CYP, cytochrome P450; 7-EROD, 7-ethoxyresorufin O-deethylation; IFN, interferon; IFU, inclusion forming units; IL, interleukin; L-NAME, N^G-nitro-Larginine methyl ester; LPS, lipopolysaccharide; NOS, nitric oxide synthase; and 7-PROD, 7-pentoxyresorufin O-dealkylation.

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trachomatis on CYP in mice appear to be mediated largely by NO.

MATERIALS AND METHODS

Infection of Mice with C. trachomatis

Two sets of experiments were performed. To determine the ability of *Chlamydiae* to induce iNOS, a group of 7 to 8-week-old BALB/c (H-2d) female mice (Charles River) were inoculated i.p. with doses ranging from 10^5 to 10^8 IFU of the C. *trachomatis* mouse pneumonitis (MoPn) biovar (strain Nigg II; American Type Culture Collection). Intraperitoneal macrophages were harvested 24 hr postinfection and lysed on ice for 20 min with a solution containing 0.14 M of NaCl, 3 mM of $MgCl_2$, 1 mM of dithiothreitol, 2 mM of phenylmethylsulfonyl fluoride, and 10 mM of Tris-HCl, pH 8.0, before they were tested by Western blot.

To assess the role of NO, a second set of mice were infected i.p. with 5×10^6 C. *trachomatis* IFU and treated with the NOS inhibitor L-NAME. The mice were injected with 20 mg/kg of L-NAME, i.p. twice daily for up to 20 days. Control animals were infected and received 200 μL of PBS following the same schedule as the animals treated with L-NAME. Another control group was mock-infected and received PBS. Blood was collected by eye puncture, and the serum was separated. Mice were euthanized at 0, 2, 4, 6, 8, 11, 14 and 20 days postinfection; their livers, spleens, and lungs were harvested and cultured, and the number of C. *trachomatis* IFU/g of tissue was determined as described before [13]. In parallel, other groups of mice were treated similarly, and their peritoneal macrophages were harvested at 2, 4, 6, and 14 days postinfection to determine their NO production.

Each group consisted of 4–6 animals, and the experiments were done in duplicate. The mice were fed rodent laboratory chow and water *ad lib*. and were kept on a 12-hr, light–dark cycle at the University of California, Irvine Vivarium.

Western Blotting

Peritoneal macrophages from the mice inoculated with 10^5 to 10^8 C. *trachomatis* IFU were harvested at 24 hr postinfection and lysed as described above. Protein lysates were separated on an 8% SDS–PAGE minigel, according to Laemmli [14], and were transferred to a nitrocellulose membrane. The membrane was blocked with 1% fetal bovine serum and incubated with rabbit polyclonal affinity purified anti-iNOS antibody (Transduction Laboratories). After washing, the blots were incubated with an anti-rabbit IgG conjugated with peroxidase, and the bands were detected with 4-chloro-1-naphthol. A positive iNOS macrophage control supplied by Transduction Laboratories, was run in parallel with the test samples.

Preparation of Hepatic Microsomes

Sections from the livers harvested at different times postinfection were kept at −80° until further testing. All subsequent steps were carried out at 4°, and microsomes were prepared as described earlier [10]. The washed microsomal pellets were resuspended in 25 mM of Tris, pH 7.25, 1.15% KCl, 20% glycerol and stored at −80°.

[NO_x] Assay

The nitrite concentration in supernatants of peritoneal macrophages collected at 2, 4, 6, and 14 days after infection with 5×10^6 C. *trachomatis* IFU was measured following stimulation with medium, or with 1 μg/mL of *Escherichia coli* LPS (serotype O111:B4) (Sigma). Briefly, 50-μL aliquots of supernatant were mixed with equal volumes of the Griess reagent (1% sulfanilamide, 0.1% napthylethylene diamine dihydrochloride, 5% H_3PO_4) and incubated at room temperature for 10 min; the O.D. was measured at 550 nm (Microtiter reader, model 450; Bio-Rad). NO_2 concentration was determined from a calibration curve prepared from $NaNO_2$ dissolved in RPMI-1640 medium with 10% fetal bovine serum. [NO_x] levels in mouse serum were measured as described earlier [15].

CYP Activity

7-EROD, 7-PROD, and 7-BROD corresponding to CYP1A and CYP2B activities in murine liver were determined by the method of Burke and Mayer [16].

Total RNA Preparation and Northern Blot

Total RNA was isolated from the frozen tissues by using UltraSpec II (BioTecx Laboratories) according to the supplied protocol. After washing with 75% ethanol and centrifugation, RNA was dissolved in FORMAzol (MRC, Inc.) and stored at −80°.

For northern blot analysis, 20 μg of total RNA was denatured (65° for 10 min), cooled, and electrophoresed on 1.5% agarose gel containing 5% formaldehyde. The denatured RNA was transferred to a Nylon+ membrane (Boehringer Mannheim) in 10× SSC (1× SSC: 0.15 M of NaCl, 15 mM of sodium citrate, pH 7.0), left overnight, and then UV-cross-linked on a UV Stratalinker 2400 (Stratagene). The membranes were prehybridized for 2 hr in QuickHyb solution (Stratagene) and hybridized overnight in a solution containing ^{32}P-labeled DNA oligomer along with 100 mg/mL of denatured salmon sperm DNA at 42°.

The following oligomer probes were used. To assess CYP2B mRNA, the sequence 5′-GGTTGGTAGCCGGT GTGA-3′ was hybridized [17]. To detect the 18s ribosomal RNA, the rat RNA-specific probe 5′-ACCTCTAGCG GCGCAATAC-3′ was utilized [18]. The probes were labeled at the 5′ end using [^{32}P]ATP (sp. act. 3000 Ci/mmol; Amersham) and T4-polynucleotide kinase (Pro-

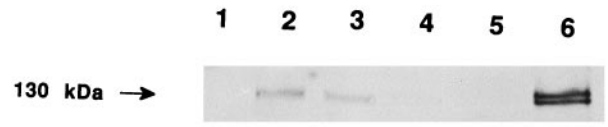

FIG. 1. Western blot analysis of iNOS in peritoneal macrophages from C. *trachomatis*-infected and control mice. Lane 1: peritoneal macrophages from mice injected with endotoxin-free PBS. Lanes 2–5: peritoneal macrophages collected at 1 day postinfection, from mice infected with 10^8, 10^7, 10^6, and 10^5 C. *trachomatis* IFU, respectively. Lane 6: positive control from Transduction Laboratories. Lysate of mouse macrophage cells was stimulated with IFN-γ (10 ng/mL) and LPS (1 μg/mL).

mega). Filters were washed twice in 2× SSPE (1× SSPE: 10 mM of phosphate buffer, pH 7.4, 150 mM of NaCl, 1 mM of EDTA) and once in 2× SSPE, 0.1% SDS for 30 min at room temperature. Autoradiography was performed by exposing the filters overnight to Kodak X-Omat XAR film at −80°. Then the filters were washed with 0.1× SSC, 0.5% SDS for 15 min at 95° and rehybridized with the 18s RNA probe as described above. Relative mRNA levels were assessed by densitometry and normalized to 18s RNA.

Protein Assay

Protein concentration was measured by the dye-binding assay of Bradford [19], with bovine serum albumin as the standard.

Materials

Anti-iNOS antibody was purchased from Transduction Laboratories. The oligonucleotide probes were custom-synthesized by Keystones. Resorufin and 7-ethoxy-, 7-pentoxy-, and 7-benzyloxyresorufin were purchased from the Sigma Chemical Co.

Statistical Analysis

The statistical analysis was done using ANOVA or Mann–Whitney, when appropriate.

RESULTS

Induction of iNOS in Peritoneal Macrophages by C. trachomatis

Figure 1 shows a Western blot with the levels of expression of iNOS in peritoneal macrophages collected from mice infected i.p. with doses ranging from 10^5 to 10^8 C. *trachomatis* IFU. Strong bands corresponding to the iNOS were detected in the peritoneal macrophages of mice inoculated with 10^8 and 10^7 IFU, while a weak band (not apparent on photograph) was observed in the macrophages from the mice injected with 10^6 IFU. No bands were detectable in macrophages from the animals inoculated with 10^5 IFU. Based on these results, we decided to use 5×10^6 C. *trachomatis* IFU for the experiments with L-NAME.

Effects of L-NAME *on a* C. trachomatis *Infection*

The results of the experiments using L-NAME to block the *in vivo* production of NO during a chlamydial infection are shown in Table 1. Overall, the number of C. *trachomatis* IFU recovered from mice infected with 5×10^6 C. *trachomatis* IFU, and treated with L-NAME, was significantly higher throughout the 20 days of the experiment than the number of IFU recovered from the control groups inoculated with PBS. For example, by days 2 and 4 postinfection, the number of IFU was already higher in the spleens (2- to 5-fold), livers (2- to 5-fold), and lungs (10-fold) of mice inoculated with L-NAME than in the controls ($P < 0.05$). By day 6 postinfection, the difference was most dramatic: 5-fold in spleen, 29-fold in the livers, and 18-fold in lungs of mice treated with L-NAME than from the PBS-inoculated controls ($P < 0.05$). By day 20 postinfection, no C. *trachomatis* was recovered from the control mice, while the organs from the L-NAME-treated animals were still culture positive.

Effects of a C. trachomatis *Infection on* $[NO_x]$ *Levels*

Peritoneal macrophages from the mice inoculated with 5×10^6 C. *trachomatis* IFU were harvested at 2, 4, 6, and 14 days postinfection, and stimulated *in vitro* with LPS to determine the NO production. As shown in Fig. 2, macrophages from mock-infected mice produced limited amounts of NO throughout the 14 days of observation whether or not they were stimulated with *E. coli* LPS. In contrast, the peritoneal macrophages from the C. *trachomatis*-infected mice had significant NO production in response to the LPS stimulus. The differences were most significant in the specimens collected at 4 and 6 days postinoculation. At 6 days postinfection, the nitrite levels in LPS-treated macrophages of the mice inoculated with C. *trachomatis* (Fig. 2, curve d) were 4.3-fold higher than in LPS-treated macrophages prepared from mock-infected mice (Fig. 2, curve b). Without LPS, macrophages from *Chlamydia*-infected mice (Fig. 2, curve c) showed only a 2.7-fold increase in NO_2 levels as compared with control mice (Fig. 2, curve a).

Similar to the dynamics of NO_2 in LPS-induced macrophages, plasma nitrite + nitrate ($[NO_x]$) levels measured in serum of mice infected with C. *trachomatis* increased steadily starting day 2 postinfection (53 ± 5.2 μM), reaching a maximum at day 8 (108 ± 9.3 μM), and then declined to almost the control level by day 14 postinfection (52 ± 5.0 μM). Total $[NO_x]$ was induced up to four-fold at day 8, as compared with the control (Fig. 3a).

Effects of a C. trachomatis *Infection on CYP Metabolism*

In contrast to the dynamics of plasma $[NO_x]$, CYP1A-dependent 7-EROD was suppressed substantially during the course of infection with up to a 51% decrease at days 6–8 postinfection in comparison to the control and restored up

TABLE 1. Effect of L-NAME on C. *trachomatis* infection

Organ	*C. trachomatis* (IFU/g tissue)							
	Day 2		Day 4		Day 6		Day 20	
	Control	L-NAME	Control	L-NAME	Control	L-NAME	Control	L-NAME
Spleen	$2.3 \pm 1.0 \times 10^5$	$12 \pm 0.3 \times 10^5$*	$4.8 \pm 1.8 \times 10^5$	$22 \pm 0.4 \times 10^5$*	$8.6 \pm 4.9 \times 10^4$	$49 \pm 2.2 \times 10^4$	0	$1.0 \pm 0.5 \times 10^2$
Liver	$9.8 \pm 4.5 \times 10^5$	$22 \pm 0.6 \times 10^5$*	$11 \pm 0.4 \times 10^5$	$53 \pm 1.3 \times 10^5$*	$2.2 \pm 0.6 \times 10^4$	$64 \pm 3.0 \times 10^4$*	0	$6.0 \pm 2.5 \times 10^2$
Lungs	$0.33 \pm 2.4 \times 10^5$	$3.1 \pm 2.5 \times 10^5$*	$1.1 \pm 0.7 \times 10^5$	$12 \pm 0.4 \times 10^5$*	$1.4 \pm 0.7 \times 10^4$	$26 \pm 1.4 \times 10^4$*	0	$12 \pm 0.4 \times 10^2$*

Mice were infected i.p. with 5×10^6 *C. trachomatis* IFU and treated with the NOS inhibitor L-NAME. The mice were injected i.p. with 20 mg/kg of L-NAME twice daily for up to 20 days. Control animals were infected and received 20 μL of PBS following the same schedule as the animals treated with L-NAME. Values are means ± SEM ($N = 3$–4).

*Significantly different from respective controls ($P < 0.05$, Mann–Whitney test).

to 77% of control level by day 14 (Fig. 3a). Similar changes were also observed in CYP2B-mediated metabolism of 7-PROD and 7-BROD (Fig. 3b). C. *trachomatis* reduced the rate of *O*-dealkylation of 7-pentoxyresorufin up to 57% of control at day 6, with this activity returning to 87% of the control level at day 11 postinfection. Analogously, 7-BROD was decreased during the course of infection up to 50% of control at day 6 and then restored towards the basal level after day 8 postinfection (75% of control).

The reduction in CYP1A activity was reversed completely in C. *trachomatis*-infected mice treated with L-NAME for 4 days (97 vs 70% of control) and significantly reversed in animals treated for 6 days (73 vs 44%, Fig. 3c). Similarly, the suppression of CYP2B-mediated metabolism was abolished completely by L-NAME on both day 4 (90 vs 64%) and day 6 (107 vs 57% of control) following infection with C. *trachomatis* and treatment with L-NAME (Fig. 3d).

Figure 4 demonstrates the changes in CYP2B mRNA in the livers of mice infected with C. *trachomatis*. Mouse-specific CYP2B9/10 shares 82% of homology with rat CYP2B [20], which allowed us to use a rat CYP2B1 probe for the detection of murine CYP2B mRNA. The northern blotting showed that C. *trachomatis* caused 88% suppression of murine hepatic CYP2B mRNA at day 8, which had not yet returned to control level by day 14 postinfection.

DISCUSSION

Several reports have shown that infections with extracellular bacteria can impair drug metabolism by inhibiting hepatic CYP microsomal monooxygenase [9]. Here we have provided evidence that a systemic infection with C. *trachomatis*, an obligate intracellular bacterium, can exert similar effects on drug-metabolizing enzymes.

The mechanisms, induced by a bacterial infection, that result in altered drug metabolism are not clearly understood. Many reports have correlated the inhibition of liver enzymes with various processes involved in the activation of macrophages such as IFN-γ, TNF-α, IL-1, and IL-6 secretion [21]. Recently, Mayer *et al.* [5] demonstrated that NO is a necessary effector molecule involved in the mechanism(s) of IFN-γ-induced inhibition of chlamydial proliferation in murine-derived McCoy cells. These results were confirmed by the work of Igietseme using both *in vitro* and *in vivo* models of *Chlamydiae* infection [4, 6].

In this *in vivo* study we showed a reversed correlation between the severity of a systemic C. *trachomatis* infection and the amounts of NO produced during the course of the infection. Furthermore, cotreatment of mice with L-NAME dramatically increased the chlamydial burden, clearly substantiating the role that NO plays in the defense against this bacteria. The mechanism(s) of NO-mediated suppression of *Chlamydiae* growth remain(s) unresolved. The cysteine-rich proteins present in the chlamydial envelope could be one of the targets for NO. Mayer *et al.* [5] have also suggested that NO could affect the replication of *Chlamydiae*, by inhibiting the activity of the enzyme ribonucleotide

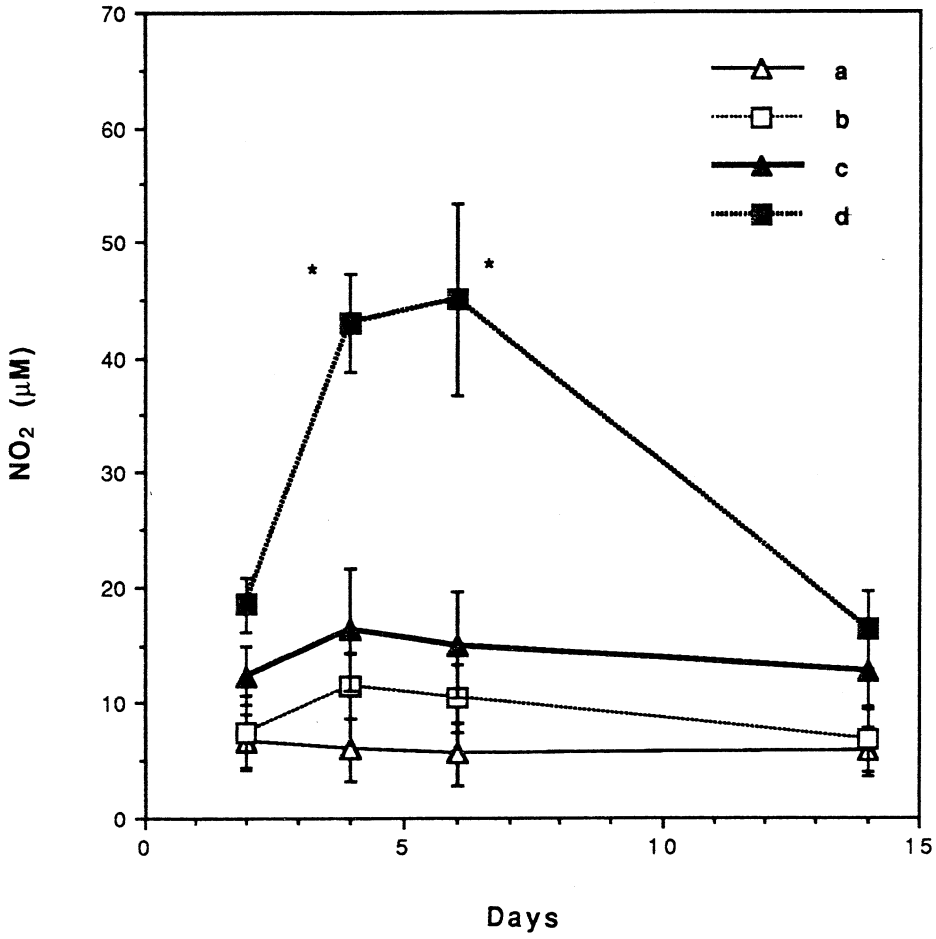

FIG. 2. Production of nitrites by peritoneal macrophages. A total of 2×10^5 peritoneal macrophages were collected from mock-inoculated (a and b) or C. *trachomatis*-infected (c and d) mice and cultured for 24 hr in the presence of *E. coli* LPS (b and d) or medium (a and c). Supernatants were collected and immediately assayed for NO_2. The results are mean ± SEM of the NO_2 concentration for triplicate cultures taken from two representative experiments. *Significantly different from the corresponding control ($P < 0.05$).

reductase, and by interfering with the production of ATP by the host cell, because this organism appears to be energy dependent.

The pathogenesis of a chlamydial infection is probably dependent on several virulence factors, including the stimulation of macrophages by chlamydial LPS [22]. Kupffer cells were shown to produce significant amounts of NO in response to an immunological stimuli [23]. Peterson and Renton [24] showed that the mechanisms of P450 inhibition involve the release of a factor from Kupffer cells. It is conceivable that NO could be the factor produced during activation of the host-defense mechanism. NO can easily penetrate the cell membrane and cause the suppression of microsomal P450s. This suppression appears to be generalized rather than isoform specific, because P450s of two distinct families, CYP1A and CYP2B, are affected to approximately the same degree. The maximal suppression for both types of P450 was observed at days 6–8 when the plasma [NO_x] levels also reached maximum. The activity was fully recovered at day 11 postinfection when nitrate concentrations were returning to basal level. This correlation, together with the fact that the specific NO inhibitor L-NAME fully protected both P450 activities from the inhibition due to infection, strongly suggests that NO is largely involved in the inhibition of murine hepatic CYP during a C. *trachomatis* infection.

Armstrong and Renton [25] also reported that CYP mRNA is lost during an active infection with *L. monocytogenes*. The present study supports these data and indicates that C. *trachomatis*, an intracellular bacteria can cause a substantial loss of CYP2B mRNA in the host liver, demonstrating that inhibition of P450 occurs also at the pretranslational level. Further work is needed, however, to determine if NO plays a similar role in other types of bacterial infections.

NO was shown recently to be important in the suppression of C4P in rats by LPS [10, 12, 26, 27]. Also, Chamulitrat *et al.* [28] found a suppression of EPR signals attributable to ferric low-spin P450/P420 peaks in the livers of mice infected with C. *parvum*. Recently, Berg–Candolfi *et al.* [29] reported that CYP3A-mediated drug metabolism in mice was suppressed during *T. gondii* infection, and this suppression was abolished significantly by *N*-acetylcysteine and N^G-monomethyl-L-arginine. However, although Donato *et al.* [30] implicated NO in inhibition of P450 by IFN-γ in human hepatocytes, Hodgson and Renton [31] failed to do that with murine P450s. It is difficult to depict the role of NO in the last study (Ref. 31) since the authors

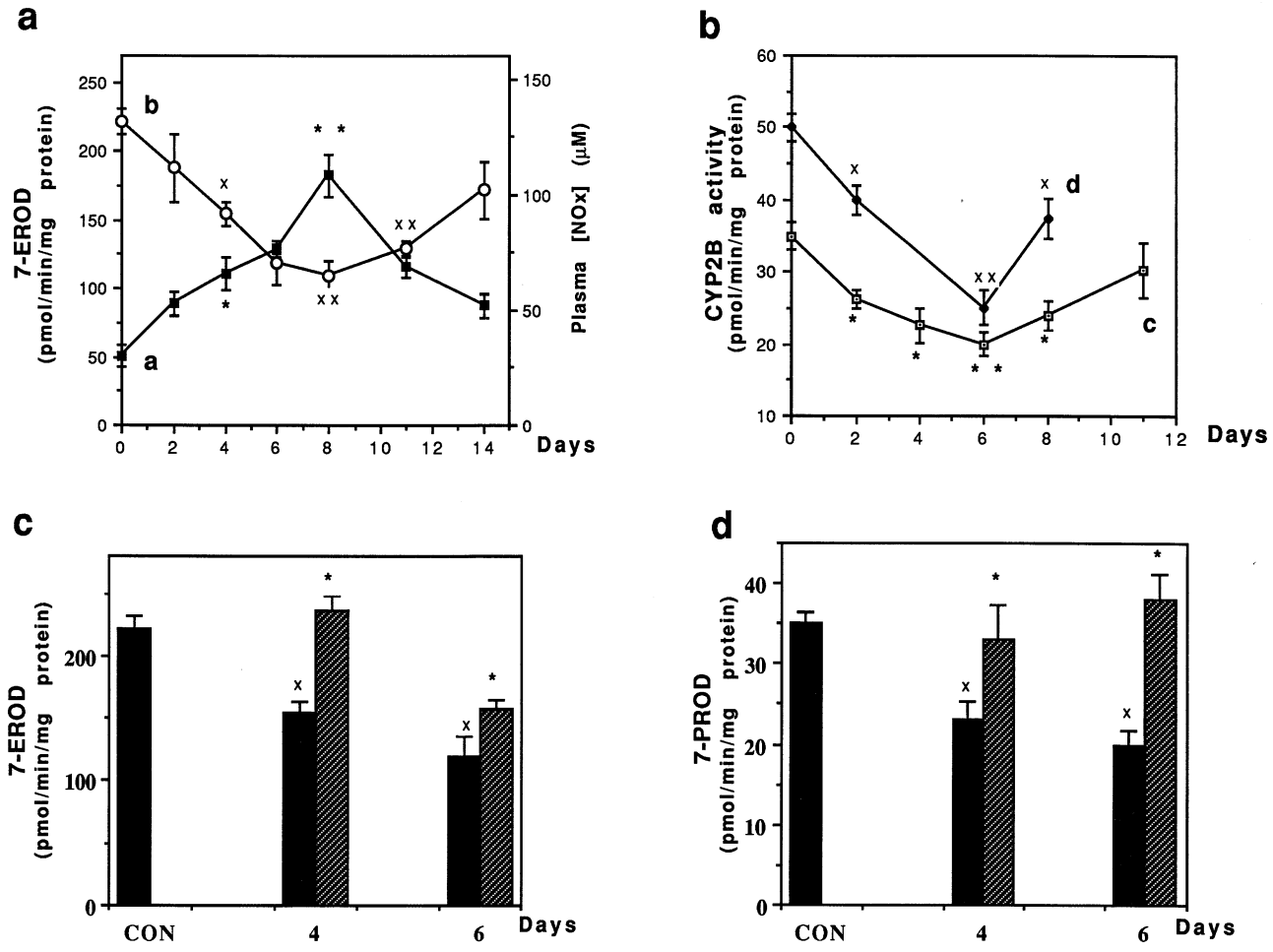

FIG. 3. (a) NO production during a systemic C. *trachomatis* infection and its effect on CYP1A-dependent metabolism in murine liver. Mice were infected i.p. with C. *trachomatis*, and serum and hepatic microsomes were prepared. The rate of NO production (curve a) was evaluated via plasma [NO_x] levels, as described in Materials and Methods. CYP1A-mediated 7-EROD (curve b) was measured as described [16] and presented as pmol/min/mg of protein. Values are means ± SEM ($N = 3–4$). Key: (*, ×) significantly different ($P < 0.05$) from the corresponding control; and (**, ××) significantly different ($P < 0.01$) from the corresponding control. (b) Inhibition of CYP2B-dependent metabolism by C. *trachomatis* in murine liver. CYP2B-mediated 7-PROD (curve c) and 7-BROD (curve d) were measured as described [16] and presented as pmol/min/mg of protein. Values are means ± SEM ($N = 3–4$). Key: (*, ×) significantly different ($P < 0.05$) from the corresponding control; and (**, ×) significantly different ($P < 0.01$) from the corresponding control. (c) Effect of C. *trachomatis in vivo* on CYP1A-mediated metabolism in murine liver in the presence (striped columns) or absence (solid columns) of the NOS inhibitor L-NAME. Groups of mice were infected with C. *trachomatis*, and two groups were treated with the NOS inhibitor L-NAME on days 4 and 6 postinfection. Hepatic microsomes were prepared and CYP1A-mediated 7-EROD activity was measured as described. Values are means ± SEM ($N = 3–4$). Key: (×) decrease in 7-EROD due to C. *trachomatis* infection in comparison with noninfected control ($P < 0.05$); and (*) protection by L-NAME against decrease in 7-EROD as compared to C. *trachomatisinfected mice* ($P < 0.05$). (d) Effect of C. *trachomatis* and L-NAME on CYP2B-mediated metabolism in murine liver. Values are means ± SEM ($N = 3–4$). Key: (×) decrease in 7-PROD due to a C. *trachomatis*infection in comparison with noninfected controls ($P < 0.05$); and (*) protection by L-NAME against decrease in 7-PROD as compared with C. *trachomatis*-infected mice ($P < 0.05$).

did not measure actual NO production in their experiments. Nevertheless, it is also possible that IFN-mediated suppression of P450, as in the case of IL-6 [32], occurs mainly through mechanisms different from that of LPS, because IFN alone appears to be a weak inducer of NO [33] and IL-6 alone does not induce it at all [32].

This is the first report showing the possible role of NO as a mediator of CYP suppression during an infection with C. *trachomatis*. The phenomenon is important, in that C. *trachomatis*-induced suppression of metabolism by CYP may prolong the duration and intensity of action of drugs, such as the antibiotic erythromycin, used for the treatment of chlamydial infections [34]. For example, erythromycin is a substrate for the CYP3A family of P450s, which, though not analyzed in the present study, has been shown to also be attenuated *in vivo* during activation of host defense [35] through an NO-dependent mechanism [12]. Such attenuation of drug metabolism and elimination can cause overdosing with consequent toxic effects.

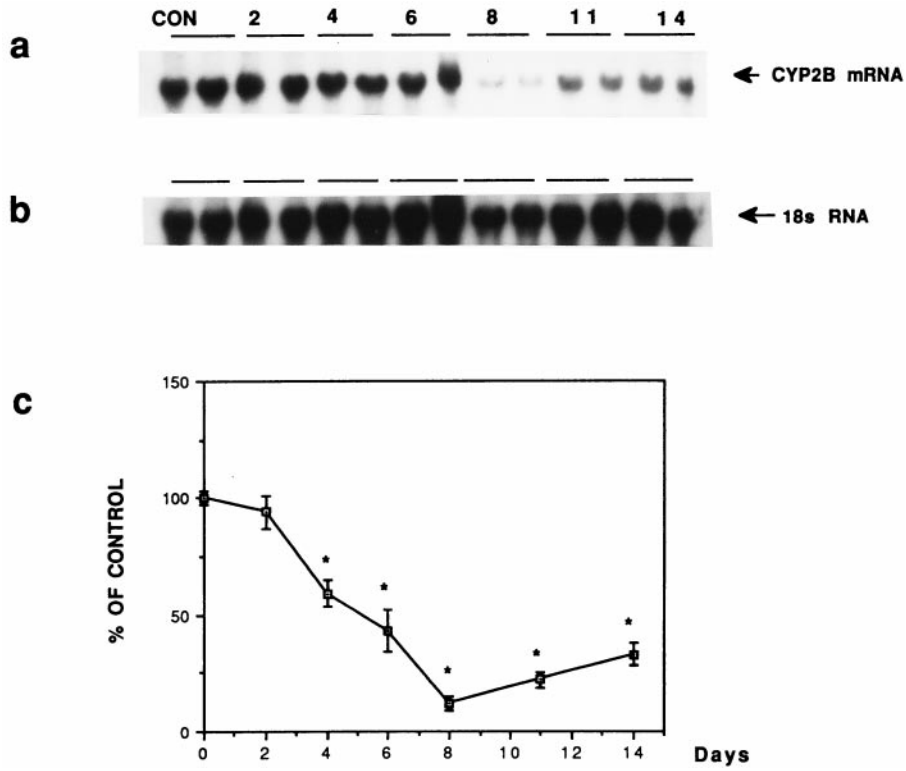

FIG. 4. CYP2B mRNA levels in the livers of mice infected with C. *trachomatis*. Animals were infected with C. *trachomatis* as described in Materials and Methods. CYP2B mRNA (a) was detected by northern blotting (on days 2–14), as described. 18s rRNA (b) was used as a loading control. Each band represents an individual animal. Blot is representative of two animals from each group of four (N = 4). (c) Densitometric analysis of CYP2B mRNA content. *Significant decrease caused by infection, relative to control ($P < 0.05$).

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