Gram-Negative Endotoxin Administration Decreases Hepatic Drug-Metabolizing Enzymes during Development in Rats

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Summary

The frequency of gram-negative infections and endotoxemia in the perinatal period prompted an investigation of the effects of endotoxin (*Escherichia coli* 026B6) on hepatic drug metabolism. Gravid female rats given injections IP with different dosages of lipopolysaccharide during late pregnancy resulted in significant depression of the liver microsomal cytochrome P-450 dependent monooxygenase activities. The acute administration of endotoxin to mothers (1.4 mg/kg on seventh day after parturition) significantly decreased the hepatic activity of aminopyrine demethylase and contents of cytochrome P-450 of suckling neonates and mothers. However, chronic administration of endotoxin (0.2 mg/kg/day for 7 days) to lactating mothers did not alter neonatal enzyme activities. When neonates themselves were given injections of endotoxin (1.0 mg/kg) at 7, 16, and 27 days of age, a significant reduction in levels of mixed function oxidase enzymes was observed. These observations suggest that the ability of mothers and neonates to metabolize drugs is significantly decreased upon exposure to endotoxin, and this demands careful evaluation of drug disposition studies in gram-negative sepsis.

Speculation

Gram-negative bacterial infections with accompanying endotoxemia represent an increasing clinical concern especially in pregnant women and children requiring a number of drugs. Endotoxin could possibly modify the host response to drug metabolism and action. This could be significant in evaluating drug toxicity of clinically used therapeutic agents used in treatment of bacterial infections. In view of this hypothesis and of well-documented liver function abnormalities, we investigated the effects of endotoxin upon the activities of rat hepatic drug-metabolizing enzymes (*in vitro*) during development.

Endotoxemia accompanying gram-negative bacterial infections is a serious life-threatening condition which frequently occurs in pregnant women, children, and adults. Endotoxin, a soluble lipopolysaccharide cell wall constituent of gram-negative bacteria, is released into body fluids during infections by these organisms. The complications of pregnancy result in an occurrence of maternal urinary tract infections and endotoxemia leading to the birth of infants with neurological defects (21). Endotoxin has been shown to induce placental damage and abortion in experimental animals (25). Recently, Aballi *et al.* (1) demonstrated significant hepatic changes in rabbit liver after a single dose of endotoxin which prepares the animal for the Shwartzman reaction. Several other investigators also demonstrated liver functions abnormalities (3, 11, 32) and diffused necrotic lesions (8, 12, 14, 19) upon endotoxin administration in several species of animals including humans. The critical step leading to the development of shock is probably the

direct injury of many types of host cells by microbial products. Bacterial endotoxins are the prototype of these substances for animal experimental investigations.

A number of therapeutic agents which are metabolized by liver are utilized in treatment of endotoxemia. However, very little is known about how the ability of the liver to metabolize drugs is altered upon administration of endotoxin. Using endotoxin derived from *E. coli*, we have previously demonstrated a decrease in the hepatic microsomal mixed-function oxidase enzyme activities of adult male (10) and female rats and also an increased hexobarbital sleeping time in male rats (34). We further report the effects of gram-negative bacterial endotoxin upon rat hepatic microsomal, drug-metabolizing enzymes during pregnancy and postnatal development.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

Sprague-Dawley rats (CD strain) were used in these experiments. Rats were date bred to sperm-positive test, or gravid females were obtained from Charles River Breeding Laboratories (Wilmington, MA) at 14 days of gestation and housed separately. Throughout pregnancy and lactation, the rats had free access to food (Purina laboratory chow; Ralston Purina Co., St. Louis, MO) and water. The endotoxin preparation was lyophilized purified lipopolysaccharide from *Escherichia coli*, serotype 026B6 (Difco Laboratories, Detroit, MI). Endotoxin was administered IP in sterile pyrogen-free isotonic saline. The control group of animals received an equal volume of saline. Experimental protocols and treatment schedule are included in "Results."

PREPARATION OF MICROSOMES AND ENZYME DETERMINATIONS

The liver microsomal preparations were made as described previously (17) and resuspended in 150 mM tris-HCl buffer (pH 7.4).

Liver microsomal cytochrome P-450 was determined by the method of Omura and Sato (23) with the assumption of an extinction coefficient of 91 mmoles⁻¹ cm⁻¹ for the carbon monoxide-cytochrome P-450 complex absorbing at 450 nM. Cytochrome *b₅* was determined with extinction coefficient of 110 mmoles⁻¹ cm⁻¹ measuring absorbance difference between 427 and 500 nM (30). An Aminco Dw-2-UV-VIS Spectrophotometer was used for cytochrome P-450 and *b₅* measurements. An *in vitro* assay of aminopyrine *N*-demethylase with the production of formaldehyde was performed according to the Nash procedure (20) as modified by Orrenius (24). Aniline (AN) hydroxylase activity was determined by the method of Dixon *et al.* (7) in which *p*-aminophenol production was monitored. Benzo(a)pyrene (BP) hydrox-

ylase activity was determined by the method of Gielen *et al.* (9). BP metabolism was measured as relative fluorescence at 523 nm with an excitation wavelength of 396 nm and compared to serial dilutions of 3-hydroxy-BP taken through the assay. The specific activity of NADPH cytochrome *c* reductase was determined as described by Phillips and Langdon (26). The rate of appearance of reduced cytochrome *c* was measured by following the absorbance change at 550 nm in a Beckman-(DU)-Guilford recording spectrophotometer, and an extinction coefficient of $19.1 \text{ mmol}^{-1} \text{ cm}^{-1}$ was used in calculating the absolute amount of cytochrome *c* reduced per min per mg of microsomal protein. UDP-glucuronyltransferase was measured as described previously (18) by estimating spectrophotometrically the amount of *p*-nitrophenol glucuronide formed using 0.9 mM *p*-nitrophenol and Triton X-100-activated microsomes. Liver microsomal protein concentration was determined by the procedure of Lowry *et al.* (16) with crystalline bovine serum albumin as the protein standard.

The data presented here were obtained from a minimum of 4 to 6 individual animals and expressed as either nmoles/mg protein (cytochrome P-450 and b_5) and nmoles/min/mg protein (aminopyrine *N*-demethylase and AN hydroxylase), mean $\pm$ S.D., or percentage of control activity.

Statistical comparisons were made using Students' *t* test; *P*'s of <0.05 were considered significant.

RESULTS

PREGNANCY

Gravid female rats were given injections IP of 0.1, 0.5, and 1.0 mg/kg (body weight) doses of endotoxin at 18 days of gestation and sacrificed 24 hr later when liver microsomal enzyme determinations were made. The data are presented in Table 1. Using 1.0 mg/kg endotoxin dose IP, 20 to 25% mortality of pregnant mothers was observed. Essentially, endotoxin administration significantly decreased the hepatic microsomal cytochrome P-450, b_5 , and mixed function oxidation reactions. However, significant dose-dependent decrease in liver microsomal mixed-function oxidase enzymes by endotoxin was not observed. In other experimental protocol, we examined the possibility that prenatal administration IP of endotoxin might decrease the liver microsomal, mixed-function oxidase enzyme activities of developing animals. Pregnant rats were given injections IP with a 0.5 mg/kg dose of endotoxin IP on 7, 11, and 15 days of gestation, and liver microsomal enzyme activities were measured postnatally on the seventh and 21st days in lactating mothers and their pups. We did not observe any significant changes in their (mothers' or pups') liver microsomal cytochrome P-450 and b_5 contents, aminopyrine *N*-demethylase, AN hydroxylase, BP hydroxylase, *p*-nitrophenol-UDP-glucuronyltransferase, or NADPH-cytochrome *c* reductase activities (data not shown).

Table 1. Decreased hepatic microsomal drug-metabolizing enzyme activities in pregnant rats by different doses of endotoxin¹

Enzyme ²	Endotoxin (mg/kg)			
	Controls	0.1	0.5 ³	1.0 ³
Aminopyrine demethylase	3.20 $\pm$ 0.48 ⁴	83 ⁵	69	65
AN hydroxylase	0.34 $\pm$ 0.08	41 ³	35	30
Cytochrome P-450	0.55 $\pm$ 0.06	71 ³	65	63
Cytochrome b_5	0.56 $\pm$ 0.03	83	86	70

¹ Endotoxin (0.1, 0.5, or 1.0 mg/kg body weight) was administered IP on the 18th day of gestation, and liver microsomal enzyme measurements were made 24 hr later.

² nmoles of product formed per mg microsomal protein per min or nmoles of cytochrome P-450 or b_5 contents per mg of microsomal protein.

³ Significant from control, $P \leq 0.05$.

⁴ Mean $\pm$ S.D.

⁵ Values are percentage of control activity.

LACTATING MOTHERS AND SUCKLING NEONATES

The purpose of this experiment was to evaluate the effects of acute or short-term chronic administration of endotoxin upon maternal and neonatal liver microsomal drug-metabolizing enzyme activities (possible endotoxin excretion via milk). Three groups of mothers were given injections immediately upon parturition with an IP dosage of endotoxin as (1) control, pyrogen-free saline only; (2) acute, endotoxin (1.4 mg/kg body weight) given on the seventh day after delivery; and (3) chronic, endotoxin (0.2 mg/kg, body weight) given daily for 7 days after delivery. In a preliminary experiment, mothers were given injections IP immediately postpartum with a 0.5 mg/kg dose of endotoxin. We observed 100% mortality within 6 to 8 hr. Therefore, the chronic dose of endotoxin (0.2 mg/kg per day for 7 days) was chosen. Lactating mothers and their neonates were sacrificed 24 hr after the last dose of endotoxin administration. Livers were removed and processed for microsomal preparations, and microsomal, mixed-function oxidase enzyme activities were determined as described earlier.

The acute administration of endotoxin significantly decreased aminopyrine *N*-demethylase activity almost by 50% and cytochrome P-450 and b_5 levels by 25% from control group. However, chronic administration of endotoxin slightly stimulated the enzyme activities. AN hydroxylase enzyme activity was not affected by both treatments of endotoxin (Table 2).

The data on the effects of acute and chronic maternal administration of endotoxin on hepatic drug metabolism of suckling neonates are presented in Table 3. Chronic administration of

Table 2. Rat hepatic microsomal drug-metabolizing enzymes decreased by acute dose but increased by chronic administration of endotoxin to lactating mothers¹

Enzyme	Endotoxin		
	Controls	Acute	Chronic
Aminopyrine demethylase ²	4.43 $\pm$ 0.05 ³	53 ⁴	114 ⁴
AN hydroxylase ²	0.36 $\pm$ 0.03	100	100
Cytochrome P-450 ⁵	0.57 $\pm$ 0.03	75 ⁴	118 ⁴
Cytochrome b_5	0.59 $\pm$ 0.05	78 ⁴	112

¹ Values are a percentage of control activity. Mothers were given injections IP postpartum with endotoxin as control-pyrogen-free saline only; acute, 1.4 mg/kg on the seventh day after delivery; and chronic, 0.2 mg/kg given daily for 7 days after delivery. Liver microsomal enzyme measurements were made 24 hr later.

² See legend to Table 1.

³ Mean $\pm$ S.D.

⁴ Significant from control, $P < 0.05$.

⁵ See legend to Table 1.

Table 3. Effects of acute and chronic maternal administration of endotoxin on hepatic drug-metabolizing enzymes of suckling neonates

Enzyme	Endotoxin ¹		
	Controls	Acute	Chronic
Aminopyrine demethylase	2.98 $\pm$ 0.3 ²	3.40 $\pm$ 1.2	2.3 $\pm$ 0.6
AN hydroxylase	0.44 $\pm$ 0.05	0.40 $\pm$ 0.07	0.40 $\pm$ 0.05
Cytochrome P-450	0.65 $\pm$ 0.07	0.57 $\pm$ 0.02 ³ (73%) ⁴	0.63 $\pm$ 0.05
Cytochrome b_5	0.51 $\pm$ 0.06	0.39 $\pm$ 0.03 (76%) ⁴	0.50 $\pm$ 0.04

¹ Acute (1.4 mg/kg) and chronic (0.2 mg/kg) endotoxin for 7 days IP to mothers on postpartum. For details, see legend to Table 2 and "Results."

² Mean $\pm$ S.D.

³ Significant from control, $P < 0.05$.

⁴ Numbers in parentheses, percentage of control. Enzyme specific activities or cytochrome P-450 and b_5 contents are as expressed under Table 1.

endotoxin to mothers did not result in any significant effect on liver microsomal, mixed-function oxidase enzymes of neonatal rats. However, an acute dose of endotoxin (1.4 mg/kg) to mothers on the seventh postpartum day significantly decreased aminopyrine *N*-demethylase activity and contents of cytochrome P-450 and *b*₅.

POSTNATAL LIFE

We further investigated the effects of endotoxin on liver microsomal, mixed-function oxidase enzymes during postnatal development. Young animals were given injections IP with a single 1.0 mg/kg dose of endotoxin on 7, 16, or 27 days of age. Animals were sacrificed 24 hr later when liver microsomal enzyme determinations were performed. Endotoxin (1.0 mg/kg) exposure IP on 16 and 27 days of age significantly increased liver-body weight ratio and decreased liver microsomal protein on 7 and 16 days of age (Table 4). Furthermore, hepatic, mixed-function oxidase enzymes and cytochrome P-450 and *b*₅ levels were significantly decreased at each time period of development. The sensitivity to the endotoxin effect (decline) on mixed function oxidase parameters decreased with developmental age (Table 5).

DISCUSSION

Recently, Nolan (22) discussed the role of endotoxins in liver injury and its hepatic and extrahepatic manifestations in certain liver diseases. Endotoxins have also been incriminated in the production of acute hepatic failure (33). The present studies demonstrate that acute administration of endotoxin during late pregnancy decreases rat hepatic microsomal mixed-function oxidase enzyme activities. However, the extent of decrease in liver microsomal, mixed-function oxidase enzymes was less conspicuous in pregnant animals as compared to previous adult animal data (10, 34). The possible explanation for this observation could be increased levels of circulating estrogens during late pregnancy protecting endotoxin effects. Numerous studies have indicated

that pharmacologic doses of steroids have a salutary effect in bacteremic and endotoxic shock in the rat (27), dog (15), and subhuman primate (31). A variety of hemodynamic and membrane-stabilizing effects have been postulated to explain the beneficial action of steroids in septic shock (28). Our observation on 100% mortality of mothers right after delivery with a dose of endotoxin (0.5 mg/kg) suggests that their susceptibility to endotoxin increases markedly compared to adult females (dose lethal to 90% of the animals, 15 mg/100 g).

We also measured placental cystein aminopeptidase (6) activity as a biochemical marker of placental function in control and endotoxin-treated (1.0 mg/kg) pregnant mothers (19 days of gestation). The specific enzyme activity (nmoles/min/mg protein) was significantly decreased by 32% from the control in the endotoxin-treated group (control, 25.5 ± 2.3 versus endotoxin treated, 17.5 ± 2.6). This preliminary data suggests possible placental damage by a low dose of endotoxin administration to mothers.

It was thought that endotoxin administration to mothers right after delivery could alter hepatic drug metabolism capacity of suckling neonates via a possible excretion of endotoxin through milk. However, chronic administration of endotoxin to mothers after delivery did not decrease the hepatic drug-metabolizing enzymes of neonates. An acute dose of endotoxin decreased mixed-function oxidase enzymes via a possible excretion of endotoxin through milk. The failure of chronic endotoxin administration in decreasing drug-metabolizing enzymes could be due to the development of nonspecific tolerance. In fact, animals can be made nonspecifically tolerant to a variety of endotoxins by giving increasingly large doses of endotoxins over a short period of time (22). Jennings *et al.* (13) made dogs tolerant over a 2-wk to 2-month period and were able to almost completely protect them against hemodynamic and biochemical effects of a normally lethal endotoxin challenge. The mechanism of this nonspecific tolerance is not well understood.

The effects of endotoxin on liver microsomal, mixed-function oxidase enzymes were more evident and decreased drug-metabolizing enzyme activities with age compared to adult male or female rats as previously reported (10, 34). This probably is related to the physiologic and biochemical maturation of the organism and age-related increase in resistance to infections.

We are investigating the mechanism of inhibition of hepatic microsomal cytochrome P-450 and dependent drug oxidation reactions by endotoxin. It is probably being converted *in vivo* to an inhibitor of mixed-function oxidase enzymes. In support of this statement, we observed that a time lapse of 6 hr is required to inhibit liver microsomal cytochrome P-450 upon *in vivo* administration of endotoxin. Furthermore, endotoxin does not directly inhibit cytochrome P-450 or aminopyrine *N*-demethylase activity on addition *in vitro* incubations. However, preincubation of endotoxin (100 µg/mg microsomal protein) with NADPH-generating system supplemented with EDTA (1 mM) and microsomes for 30 min, significantly decreases the level of cytochrome P-450 and aminopyrine *N*-demethylase activity (34) after accounting for lipid peroxidation by NADPH itself (29). These observations suggest that liver microsomal membrane lipid peroxidation may be enhanced by endotoxin. The mechanism for the depression of hepatic cytochrome P-450 by endotoxin has been suggested by Bissell and Hammaker (4, 5). They observed marked enhancement of the degradation of cytochrome P-450 heme by reversible dissociation of heme moiety from its apoprotein and by mixing with exogenously synthesized heme which regulates δ -amino-levulinic acid synthetase activity.

CONCLUSION

Acute administration of gram-negative bacterial endotoxin during pregnancy and postnatal period decreases rat liver drug-metabolizing enzymes. These observations suggest clinically possible adverse drug reactions and demand a careful evaluation of drug disposition studies in neonates with gram-negative infections.

Table 4. Increase in liver/body weight ratio and decrease in microsomal protein¹ by endotoxin² during postnatal development

Treatment	Postnatal age (days)					
	7		16		27	
	L/B ³	Protein	L/B	Protein	L/B	Protein
Control	3.8	15.0 ⁴	3.2	18.6	4.9	18.4
Endotoxin	4.4	11.2 ⁵	3.5 ⁵	16.7 ⁵	5.7 ⁵	17.2
% of change	117	25	108	11	117	7

¹ Values are mg microsomal protein per g of wet liver weight.

² Endotoxin dose (1.0 mg/kg IP) given 24 hr before sacrifice.

³ L/B, liver/body weight ratio.

⁴ Mean $\pm$ S.D.

⁵ Significant from control values, $P \leq 0.05$.

Table 5. Decrease in rat hepatic, mixed-function oxidase enzymes during postnatal development by endotoxin¹

Enzyme	Postnatal age (days)		
	7	16 ²	27
Aminopyrine demethylase	78 ²	48	34 ²
AN hydroxylase	N.D. ³	28	6
Cytochrome P-450	56 ²	32	25 ²
Cytochrome <i>b</i> ₅	32 ²	26	20 ²

¹ Values are a percentage of control activity. Endotoxin dose (1.0 mg/kg IP) given 24 hr before sacrifice (see "Results").

² Significantly different from control, $P < 0.05$.

³ N.D., not detectable.

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