The suppressive effects of lipopolysaccharide-induced acute phase response on hepatic cytochrome P450-dependent drug metabolism in rabbits

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The acute phase response (APR) was induced by five separate intravenous (i.v.) injections of *Escherichia coli* lipopolysaccharide (LPS, 17 μg/kg each time) in rabbits, with intervals of 1 h. This model was used to study the effects of APR on the activities of hepatic microsomal cytochrome P450 (CYP)-dependent enzyme including drug metabolism. Five female rabbits were included in each of four groups, a control group and three LPS-treated groups (group I, II and III). The rabbits of the control, group I, II and III were killed at 1, 1, 3 and 7 days after saline (control only) or the LPS injection, respectively. The APR was confirmed by increases in rectal body temperature, plasma concentrations of interleukin-6 and C-reactive protein (CRP). Pharmacokinetics of antipyrine before death were examined in every group. Antipyrine was administered (5 mg/kg) at 24 h (control and group I), 3 days (group II) and 7 days (group III) after the first LPS injection. Total body clearance (Cl_{tot}) of antipyrine tended to decrease in group I. All the livers were excised for measuring CYP-dependent activities. Total CYP content and several CYP-dependent activities (aminopyrine N-demethylation, aniline 4-hydroxylation and caffeine 3-demethylation) decreased in group I. The maximum velocity (V_{max}) values of those enzymes, and the amount of CYP1A1/1A2 and CYP2E1 apoproteins appeared to decrease. Michaelis constant (K_m) values of those enzymes were not affected by the APR. Rectal body temperature recovered to normal at 3 days after the first LPS injection in group II and III. The concentration of CRP, albumin, total CYP content and the plasma clearance of antipyrine returned to the control levels at 7 days after the first LPS injection. These results suggest that the metabolism of drugs, including CYP-dependent drug metabolizing activity, is suppressed markedly in incipient APR induction in rabbits, and the drug metabolizing capacity is returned to normal at 7 days after APR induction.

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INTRODUCTION

Pathophysiological conditions because of infection, inflammation, tissue damage, neoplastic growth, or immunological disorder induce a large number of metabolic changes, referred to collectively as acute phase response (APR) (Kushner, 1982; Van Miert, 1991, 1995). The APR is characterized clinically by several events including fever, increased lassitude and loss of appetite (Van Miert, 1991, 1995). The APR includes an increase in synthesis and secretion of acute phase hepatic proteins (Fey & Gauldie, 1990; Ballmer *et al.*, 1991) and impairment of hepatic drug metabolism (Renton, 1986; Monshouwer *et al.*, 1995a, 1995b, 1996).

Bacterial lipopolysaccharide (LPS) which is released during gram-negative bacterial infection will induce APR, which leads to production of proinflammatory cytokines, including tumour necrosis factor-α, interleukins and interferons. The excessive cytokines directly suppress hepatic microsomal cytochrome P450 (CYP)-dependent enzyme (Wright & Morgan, 1991; Chen *et al.*, 1992; Abdel-Razaak *et al.*, 1993). It has been reported that the clearance of several drugs was impaired by LPS in humans (Shedlofsky *et al.*, 1994). The suppression of CYP-dependent activities during the APR affected the plasma disposition of certain drugs. The relationship between the disposition of antipyrine and CYP-dependent activities was examined in APR-induced pigs (Monshouwer *et al.*, 1996).

Several studies have been conducted to elucidate the association between CYP-dependent drug metabolism and APR as the model of disease (Proulx & Du Souich, 1995; Monshouwer *et al.*, 1995b, 1996; Mills *et al.*, 1997). However, there are no reports concerning the change of CYP-dependent drug metabolism during the

recovery from APR to our knowledge. To elucidate the alterations of CYP-dependent drug metabolism during the recovery period from APR is important in establishing a proper dosage regimen for animals under pathophysiological conditions. Moreover, rabbits are now one of the most popular pet animals in Japan, and also require treatment by veterinarians. More than 200 000 rabbits are estimated to be reared from the annual total food sales for rabbits.

In the present study, we investigated the effect of LPS-induced APR on drug disposition in rabbits, including CYP-dependent drug metabolism, and the change in ability to metabolize drug during the recovery from APR. The extent of CYP-dependent activities was compared between rabbits and other species.

MATERIALS AND METHODS

Drugs and chemicals

Escherichia coli lipopolysaccharide (0111:B4, LPS), NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, 1,3-dimethylxanthine, 7β-hydroxypropyltheophylline, and polyoxyethylene (20) cetyl ether were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Antipyrine, phenacetin, caffeine, aminopyrine, aniline, *p*-aminophenol hydrochloride, *p*-nitrophenol, and UDP-glucuronic acid (UDPGA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyclonal goat antibodies against rabbit CYP1A1/A2 and CYP2E1 were obtained from Oxford Biochemical Research, Inc. (Oxford, MI, USA). 5-Bromo-, 4-chloro-, 3-indole-toluidine phosphate (BCIP) and nitroblue tetrazolium chloride (NBT) were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Animals and treatments

Twenty female rabbits (Japanese white), 4-months old weighing 2.3–2.9 kg were obtained from Nippon Institute for Biological Science (Yamanashi, Japan). All animals were allowed free access to food and water during the study. The animals were housed in individual stainless steel cages at 20 ± 1°C on a 12 h light/dark cycle and a relative humidity between 30% and 60%. These rabbits were randomly divided into a control group and three LPS-treated groups. Five females each were assigned to the control, group I, II and III, and were killed at 1, 1, 3 and 7 days after saline (control only) or LPS injection, respectively. APR was produced by the method described in Monshouwer *et al.* (1996). LPS dissolved in saline (0.1 mg/mL) was administered intravenously. A total dose of 85 μg/kg was given as five separate injections of 17 μg/kg each, with intervals of 1 h. Control rabbits received an equal volume of saline at the same time intervals. This experiment was approved by the animal care and use committee of Tokyo University of Agriculture and Technology.

Rectal body temperatures were measured and blood samples were collected from the articular vein at 20 h before, and 0, 2, 4, 8 and 24 h after the first LPS injection in the control group and in group I. In group II, they were carried out at 30 h, 48 h and 3 days after the first LPS injection. In group III, they were carried out at 7 days after the LPS injection. The heparinized plasma for the determination of interleukin-6 (IL-6), C-reactive protein (CRP) and albumin (ALB) plasma levels were separated from blood samples by centrifugation (1 500 × ***g***, 15 min) at 4 °C and stored at −70 °C until analysis.

The rabbits received antipyrine at the dose of 5 mg/kg intravenously (dissolved in saline, 20 mg/mL) at 24 h after the first LPS injection in the control group and in group I. In group II and III, it was administered at 3 and 7 days after the LPS injection, respectively. Blood samples were collected from the auricular vein at 0.25, 0.5, 1, 2 and 3 h after the antipyrine injection. The heparinized plasma for the determination of plasma concentrations of antipyrine were separated by centrifugation (1 500 × ***g***, 15 min) at 4 °C and stored at −70 °C until analysis.

The rabbits were killed under anaesthesia by intravenous injection with thiopental sodium (25 mg/kg) 1 day after the LPS injection in the control group and in group I, and 3 and 7 days after the LPS injection in group II and III, respectively. The liver was immediately excised, flushed with cold 1.15% potassium chloride containing 0.2 mM of ethylenediaminetetraacetic acid (disodium salt), 0.1 mM of dithiothreitol, 0.1 mM of phenylmethylsulfonyl fluoride and 20% glycerol (homogenate buffer), and quickly frozen (−70 °C) for measuring CYP content and CYP-dependent activities.

Plasma biochemical analysis

CRP and ALB were determined by immuno-nephelometry (Clinimate CRP, Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) and a bromcresol green method (Clinimate ALB, Daiichi Pure Chemicals Co. Ltd.), respectively, using an automated analyzer (Model 7060, Hitachi Ltd., Tokyo, Japan). IL-6 was determined by an enzyme-linked immunosorbent assay (ELISA) system for mouse IL-6 (Amersham International plc., Buckinghamshire, UK) using a microplate photometer (Model MTP-22, Corona Electric Co., Ltd., Ibaragi, Japan).

Antipyrine analysis

Antipyrine plasma concentrations were analysed by a modified high-performance liquid chromatography (HPLC) method described by Teunissen *et al.* (1983b). Briefly, 4 M sodium hydroxide (0.1 mL) and 0.1 mL of 200 μg/mL of phenacetin (dissolved in mobile phase as internal standard) were added to 0.2–1 mL of plasma. After 5 mL of dichloromethane/*n*-pentane (1/1, v/v) was added, the solution was mixed on a shaker for 5 min and centrifuged at 1500 × ***g*** for 5 min. The organic layer was collected and evaporated to dryness under nitrogen at 30 °C. The residue was dissolved in 0.5 mL of mobile phase. This solution (20 μL) was injected into a HPLC system.

The HPLC system consisted of a Model 79852A pumping system, a model 79853A variable wavelength detector set at 254 nm and a model 79855A autosampler from Hewlett-Packard Co. (Palo Alto, CA, USA). The chromatographic data were processed by a model LC100W/F work station (Yokogawa Analytical Systems, Tokyo, Japan).

Samples were chromatographed at ambient temperature (22–25°C) on a 4.6 mm i.d. × 250 mm stainless steel column packed with TSKgel ODS–120T (5 μm; Tosoh Co., Tokyo, Japan). The mobile phase was a mixture of 35% acetonitrile and 65% distilled water, adjusted to pH3 with phosphoric acid, at a flow rate of 1.0 mL/min.

A one-compartment open model was used for pharmacokinetic analysis of antipyrine. The area under the concentration-time curve (*AUC*) from time zero to infinity, the elimination rate constant (k_{el}), the apparent volume of distribution (V_d), and the mean residence time (*MRT*) were obtained by using the computer program MULTI (Yamaoka *et al.*, 1981). Total body clearance (Cl_{tot}) and terminal elimination half-life ($t_{½}$) were calculated as follows: Cl_{tot} = Dose/*AUC* and $t_{½} = 0.693/k_{el}$.

Microsomal assays

Hepatic microsomes were prepared as follows: The liver was homogenized in 5 volumes of the homogenate buffer using a Polytron® (Kinematica, Littau/Luzern, Switzerland). The homogenate was centrifuged at 10 000 × ***g*** for 15 min and the supernatant was ultracentrifuged at 105 000 × ***g*** for 70 min. The microsomal pellet was resuspended in the homogenate buffer and stored at −70 °C until use.

The total content of CYP in microsomes was measured by the method of Omura & Sato (1964) using as extinction coefficient 91/mM/cm. Total concentration of microsomal protein was determined by the method of Bradford (1976) using bovine serum albumin as standard. All CYP-dependent activity assays were performed at 37 °C in a reaction mixture (2.5 mL) containing 0.1 M sodium-potassium buffer (pH 7.4), 0.4 mM NADP, 8 mM glucose-6-phosphate, 0.5 unit/mL glucose-6-phosphate dehydrogenase, 5 mM $MgCl_2$ and microsomes (≈1 mg protein).

Aminopyrine N-demethylase (AMD) and aniline 4-hydroxylase (ANH) activities were determined as the formation of formaldehyde according to Nash (1953) and as the formation of 4-aminophenol according to Kato & Gillette (1965), respectively. The end products were measured spectrophotometrically (Model DU-65, Beckham Instruments, Inc., Fullerton, CA, USA) at 415 nm and 620 nm, respectively.

The demethylation of caffeine to 1,3-dimethylxanthine (13X) was determined by the modified method of Monshouwer *et al.* (1996). Caffeine was added to the mixture at a final concentration of 2 mM, and the mixture (1 mL) was incubated for 30 min. After incubation, 30% trichloroacetic acid (0.2 mL) was added to the mixture to stop the reaction, and 7β-hydroxypropyltheophylline (80 nmol) was added as the internal standard. The sample was centrifuged at 1 500 × ***g*** for 5 min, and the supernatant was applied to a BOND ELUT C18 column (Analytichem International, Habor City, CA, USA). The BOND ELUT C18 column was rinsed twice with 1 mL of methanol (MeOH), and was then rinsed twice with 1 mL distilled water before use. The sample was then loaded onto the column. After applying the sample, the column was washed twice with 1 mL of distilled water, and was eluted twice with 0.4 mL of MeOH. The eluate was evaporated to dryness under nitrogen at 40 °C, and the residue was dissolved in 0.4 mL of mobile phase. This solution (20 μL) was injected into the HPLC system for determining 13X. Samples were chromatographed at 40 °C and monitored at 278 nm. The mobile phase was a mixture of 15% acetonitrile and 85% distilled water (adjusted to pH3 with phosphoric acid) at a flow rate of 1.0 mL/min.

V_{max} and K_m values of AMD, ANH and caffeine 3-demethylase (CAD) in the control group and in group I were measured by pooled microsomes in each group.

UDP-glucuronosyl transferase (UDPGT) activity toward *p*-nitrophenol was determined colorimetrically by the modified method of Isselbacher *et al.* (1962). Briefly, the reaction mixture (0.2 mL) consisted of 100 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM *p*-nitrophenol, 5 mM UDPGA, and microsomal preparation (≈ 1 mg protein) containing 0.05% polyoxyethylene (20) cetyl ether (Brij-58). After a pre-incubation of 1 min, the reaction was started by addition of 5 mM UDPGA and incubated for 30 min at 37 °C. The reaction was stopped by adding 1 mL of 0.5 M trichloroacetic acid. The mixture was kept on ice for 30 min and was centrifuged at 1 500 × ***g*** for 10 min. The supernatant (0.5 mL) was mixed with 2.5 mL of 0.315 M KOH, and the absorbance was measured at 400 nm.

Western blot

The relative concentrations of various CYP apoproteins in hepatic microsomes were measured by Western blot analysis in the control group and in group I. Proteins were separated by polyacrylamide gel electrophoresis (10% polyacrylamide) in the presence of SDS. After electrophoresis, the proteins were blotted onto PVDF membranes (Bio-Rad Laboratories, Hercules, CA, USA). The CYP apoproteins were detected with goat antibodies against rabbit CYP1A1/A2 and CYP2E1 using anti-goat IgG conjugated with alkaline phosphatase (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) as a secondary antibody. CYP apoproteins were stained by a colour mixture of BCIP and NBT.

Statistical analysis

In group I, data were first analysed by the *F*-test (Snedecor & Cochran, 1980) for homogeneity of variance with significant levels of 5%. If the test revealed homogeneity of variance, a comparison was made between the LPS-treated and control groups using the Student's *t*-test (Snedecor & Cochran, 1980). In the case of heterogeneity of variance, a comparison was made between the LPS-treated and control groups using the Aspin-Welch *t*-test (Snedecor & Cochran, 1980). Differences were considered significant at $P < 0.05$.

In group II and III, the data obtained for rectal body temperature, and plasma concentrations of CRP and ALB were compared with the data for pre-LPS treatment. In the case of antipyrine pharmacokinetics, total CYP content and CYP-dependent activities, comparison was made with the control group. Data were first analysed by Bartlett's test (Snedecor & Cochran, 1980) for homogeneity of variance with significant levels of 5%. If the test revealed homogeneity of variance, the data were assessed by one way analysis of variance. If the levels

were significant at $P < 0.05$, a comparison was made between the LPS-treated groups and the control group using a two-tailed Dunnett's multiple comparison test (Dunnett, 1955, 1964) with significant levels of 5%. In the case of heterogeneity of variance, the data were analysed by the Kruskal-Wallis test (Kruskal & Wallis, 1952). When the levels were significant at $P < 0.05$, a comparison was made between the LPS-treated groups and the control group using two-tailed nonparametric Dunnett's test (Dunnett, 1955, 1964) on ranks with significant levels of 5%.

RESULTS

APR induction and recovery from APR

Anorexia, corneal hyperaemia and increased lassitude were observed in the LPS-treated animals. These abnormal signs were not observed 3 days after LPS injection. After the first injection of LPS to rabbits, rectal body temperature increased rapidly, and returned to pre-treatment levels at 3 days after LPS injection (Table 1).

Plasma concentrations of IL-6 after the first injection of LPS to rabbits raised rapidly, and peak concentration was observed 2 h after the first LPS injection (Fig. 1). The injection of LPS caused increased CRP in rabbit plasma (Table 2). CRP concentrations returned to pre-treatment levels by 7 days after injection.

Plasma concentrations of ALB gradually decreased until 3 days after LPS injection (Table 3). At 7 days after injection, ALB plasma concentrations in the LPS-treated group had returned to the pre-treatment level.

Antipyrine pharmacokinetics

Plasma time–concentration curves of antipyrine in LPS-treated and control groups are shown in Fig. 2. Antipyrine plasma concentrations decreased monoexponentially. The elimination

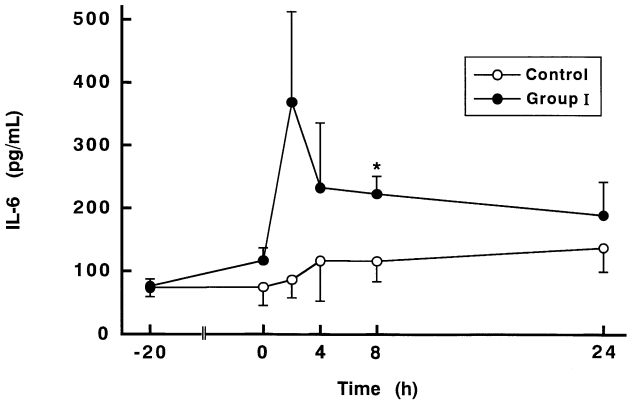

Fig. 1. Plasma IL-6 concentrations following saline or LPS injection in rabbits. LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1h (0 h, 1 h, 2 h, 3 h and 4 h). Blood samples were collected at 20 h before and at 0 h, 2 h, 4 h, 8 h and 24 h in control group and group I after the first LPS injection. Values represent mean ± SEM of group I ($n = 5$) and control group ($n = 5$) rabbits. *Significantly different from control group at $P < 0.05$.

Table 1. Rectal body temperatures (°C) in control and LPS-treated rabbits

	Pre-LPS treatment	After LPS injection								
Group	−20 h	0 h	2 h	4 h	8 h	24 h	30 h	48 h	3 day	7 day
Control	38.4 ± 0.1	38.4 ± 0.1	38.4 ± 0.2	38.5 ± 0.2	38.6 ± 0.1	39.0 ± 0.1	–	–	–	–
I	38.5 ± 0.2	38.6 ± 0.2	39.7* ± 0.2	39.8* ± 0.2	39.0 ± 0.2	40.0* ± 0.1	–	–	–	–
II	38.5 ± 0.2	–	–	–	–	–	40.4† ± 0.1	38.9† ± 0.2	38.6 ± 0.1	–
III	38.5 ± 0.1	–	–	–	–	–	–	–	–	38.7 ± 0.1

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h (0 h, 1 h, 2 h, 3 h, and 4 h) in group I, II and III. Control rabbits were given saline at the same time intervals. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control group ($n = 5$) rabbits. *Significantly different from control group values at $P < 0.05$; †Significantly different from pre-LPS treatment values at $P < 0.05$.

Table 2. Plasma concentrations of CRP (mg/dL) in control and LPS-treated rabbits

	Pre-LPS treatment	After LPS injection								
Group	−20 h	0 h	2 h	4 h	8 h	24 h	30 h	48 h	3 day	7 day
Control	0.32 ± 0.05	0.81 ± 0.07	1.19 ± 0.33	1.04 ± 0.27	1.49 ± 0.28	2.60 ± 0.53	–	–	–	–
I	0.21 ± 0.05	1.30 ± 0.45	1.43 ± 0.49	1.59 ± 0.56	1.83 ± 0.48	7.20* ± 0.35	–	–	–	–
II	0.16 ± 0.01	–	–	–	–	–	1.83† ± 0.27	2.28† ± 0.20	2.19† ± 0.44	–
III	0.15 ± 0.02	–	–	–	–	–	–	–	–	0.04 ± 0.02

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h (0 h, 1 h, 2 h, 3 h, and 4 h) in group I, II and III. Control rabbits were given saline at the same time intervals. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control group ($n = 5$) rabbits. *Significantly different from control group values at $P < 0.05$; †Significantly different from pre-LPS treatment values at $P < 0.05$.

Table 3. Plasma concentrations of ALB (g/dL) in control and LPS-treated rabbits

	Pre-LPS treatment	After LPS injection								
Group	−20 h	0 h	2 h	4 h	8 h	24 h	30 h	48 h	3 day	7 day
Control	2.25 ± 0.06	2.31 ± 0.06	2.30 ± 0.06	2.25 ± 0.04	2.26 ± 0.06	2.19 ± 0.05	–	–	–	–
I	2.13 ± 0.10	2.21 ± 0.08	2.25 ± 0.06	2.11 ± 0.07	2.08 ± 0.10	1.92* ± 0.10	–	–	–	–
II	2.20 ± 0.05	–	–	–	–	–	1.84† ± 0.05	1.85† ± 0.03	1.71† ± 0.02	–
III	2.29 ± 0.03	–	–	–	–	–	–	–	–	2.15 ± 0.10

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h (0 h, 1 h, 2 h, 3 h, and 4 h) in group I, II and III. Control rabbits were given saline at the same time intervals. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control group ($n = 5$) rabbits. *Significantly different from control group values at $P < 0.05$; †Significantly different from pre-LPS treatment values at $P < 0.05$.

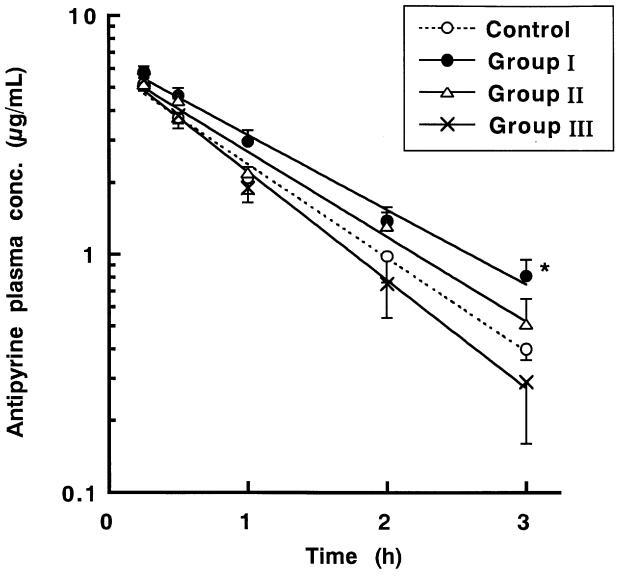

Fig. 2. Plasma antipyrine time–concentration curves of LPS-treated and control rabbits. LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h. Antipyrine (5 mg/kg) was given intravenously at 1 day after the saline injection (control), and 1 day (group I), 3 days (group II) and 7 days (group III) after the LPS injection. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control group ($n = 5$) rabbits. *Significantly different from control group at $P < 0.05$.

half-life of groups I and II appeared to be longer than that of the control.

Pharmacokinetic parameters for antipyrine are shown in Table 4. In group I, the *AUC* was significantly higher (1.5-fold) than that in control animals. Although a significant difference was not observed, Cl_{tot} appeared to decrease by 33% of the control, and $t_{½}$ and *MRT* appeared to increase by 1.4- and 1.5-fold, respectively, compared to those of the control group values. V_d was not significantly different between the LPS-treated groups and the control group. There was a non-significant trend towards a decrease in Cl_{tot} and an increase in $t_{½}$ and *AUC* observed in group II. Those parameters in the group III were almost similar to the control.

Total CYP content and hepatic microsomal activities

The changes in total CYP content in hepatic microsomes at 7 days after the LPS injection are shown in Fig. 3. Total CYP content was significantly decreased in groups I and II, with a substantial decrease found in group II. CYP content in group III was similar to that in the control group. The effects of the APR on several CYP-dependent enzyme and *p*-nitrophenol UDPGT activities are presented in Table 5. ANH and CAD activities in group I were significantly lower than those in the control group. AMD and ANH activities in group II were significantly lower than those in the control. UDPGT activity toward *p*-nitrophenol was not significantly different between the LPS-treated and control groups.

Table 4. Pharmacokinetic parameters of antipyrine in control and LPS-treated rabbits

		LPS		
Pharmacokinetic parameters	Control ($n = 5$)	Group I ($n = 5$)	Group II ($n = 5$)	Group III ($n = 5$)
Cl_{tot} (L/h/kg)	0.86 ± 0.08	0.58 ± 0.07	0.76 ± 0.08	0.96 ± 0.11
$t_{½}$ (h)	0.63 ± 0.06	0.88 ± 0.06	0.77 ± 0.04	0.58 ± 0.13
V_d (L/kg)	0.75 ± 0.03	0.75 ± 0.05	0.78 ± 0.03	0.69 ± 0.09
AUC (μg·h/mL)	6.05 ± 0.56	9.14 ± 1.02*	6.87 ± 0.80	5.46 ± 0.88
MRT (h)	1.36 ± 0.09	2.10 ± 0.45	1.32 ± 0.18	1.03 ± 0.15

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h. Antipyrine (5 mg/kg) was given intravenously at 1 day after the saline injection (control), and 1 day (group I), 3 days (group II) and 7 days (group III) after the LPS injection. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control groups ($n = 5$) rabbits. *Significantly different from control group values at $P < 0.05$.

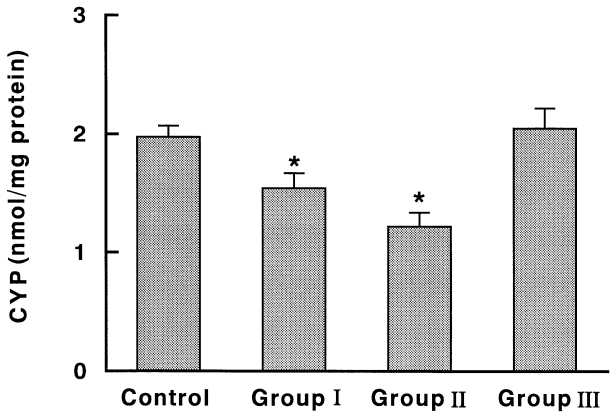

Fig. 3. Total content of cytochrome P450 following saline or LPS injection in rabbits. LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h. The liver excised at 1 day after the saline injection (control), and 1 day (group I), 3 days (group II) and 7 days (group III) after the LPS injection. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control group ($n = 5$) rabbits. *Significantly different from control group at $P < 0.05$.

V_{max} and K_m values of the CYP-dependent enzymes which were downregulated in group I are shown in Table 6. The V_{max} values of AMD, ANH and CAD activities in group I appeared to be lower than those in the control group. V_{max} values of those appeared to be decreased by 43%, 48% and 47% of the control group, respectively. K_m values for AMD, ANH and CAD in group I were almost similar to control.

Western blot analysis

Detection of apoproteins by several antibodies against CYP subfamilies on Western blot analysis of the hepatic microsomal preparations from the group I and control rabbits were shown in Fig. 4. Amounts of the apoprotein detected by anti-rabbit CYP1A1/1A2 and CYP2E1 appeared to decrease in group I animals.

Table 6. V_{max} and K_m values of the several CYP-dependent enzymes in group I

Enzymes		V_{max} (nmol/mg protein/min)	K_m (mM)
Aminopyrine N-demethylase	Control	20.3	3.31
	LPS	11.6	3.92
Aniline 4-hydroxylase	Control	1.34	0.44
	LPS	0.70	0.57
Caffeine 3-demethylase	Control	87.6×10^{-3}	1.02
	LPS	46.6×10^{-3}	0.91

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h. The livers were excised 1 day after the saline (control) and the LPS (group I) injections.

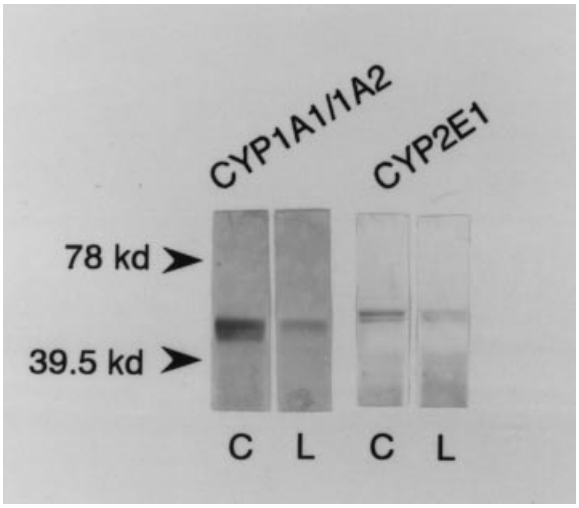

Fig. 4. Western blot showing repression of CYP1A1/A2 and CYP2E1 apoproteins from microsome. Microsomal protein (10 μg) from LPS-treated (L) and control (C) rabbits were subjected to polyacrylamide gel electrophoresis in the presence of SDS and blotted on to a PVDF membrane. The membranes were probed with anti-rabbit antibodies to CYP1A1/1A2 and CYP2E1 as described in Materials and methods.

Table 5. Effect of LPS on hepatic CYP-dependent enzyme activities and UDP-glucuronosyl transferase

Enzymes	Control ($n = 5$)	Group I ($n = 5$)	Group II ($n = 5$)	Group III ($n = 5$)
Aminopyrine N-demethylase (nmol/mg protein/min)	17.6 ± 2.2	11.8 ± 0.6	9.5 ± 0.7*	14.9 ± 0.7
Aniline 4-hydroxylase (nmol/mg protein/min)	1.31 ± 0.12	0.73 ± 0.13*	0.80 ± 0.05*	1.13 ± 0.10
Caffeine 3-demethylase (pmol/mg protein/min)	51.8 ± 3.6	31.4 ± 5.6*	40.2 ± 5.4	50.2 ± 3.6
UDP-glucuronosyl transferase (nmol/mg protein/min)	54.0 ± 5.0	53.9 ± 4.5	51.5 ± 2.7	66.4 ± 3.4

LPS was administered intravenously by five separate injections of 17 μg/kg with intervals of 1 h. The livers were excised at 1 day after the saline injection (control), and 1 day (group I), 3 days (group II) and 7 days (group III) after the LPS injections. Values represent mean ± SEM of group I ($n = 5$), group II ($n = 5$), group III ($n = 5$), and control groups ($n = 5$) rabbits. *Significantly different from control group values at $P < 0.05$.

DISCUSSION

APR was induced by the intermittent LPS injection in rabbits as shown by increases in rectal body temperatures, IL-6 and CRP plasma levels. The APR induced a significant decrease in total hepatic microsomal CYP content and in several CYP-dependent activities. Total CYP content and antipyrine plasma clearance recovered at 7 days after the LPS injection.

Proinflammatory cytokine, which is known to be involved in regulating the host defense against infection and in suppressing CYP-dependent activities, includes tumour necrosis factor-α, interleukin-1α, IL-6, and interferons (Andus *et al.*, 1988; Dinarello, 1989). These cytokines have been reported to act directly on CYP gene expression in the liver (Wright & Morgan, 1991; Chen *et al.*, 1992; Abdel-Razaak *et al.*, 1993). CRP is a type of acute phase proteins. The concentration rises rapidly during APR in humans and rabbits (Kushner, 1982; Fey & Gauldie, 1990). In the present study, IL-6 and CRP raised rapidly after the LPS injection. Thus, APR induction was confirmed by increases of CRP and IL-6 concentrations in plasma as well as rectal body temperatures.

CYP-dependent enzyme activities, apoprotein and/or mRNA levels of CYP subfamilies (1A, 2A, 2B, 2C, 2D, 2E and 3A) have been shown to decrease in response to induced APR in rats (Morgan, 1989, 1993; Sewer *et al.*, 1996), in pigs (Monshouwer *et al.*, 1996), in rabbits (Proulx & Du Souich, 1995), and in horses (Mills *et al.*, 1997). We showed that hepatic microsomal AMD (CYP2C3), ANH (CYP2E1) and CAD (CYP1A2) activities were reduced during the APR in rabbits, and the suppression of total CYP content, AMD and ANH in LPS-treated rabbits were almost similar to those of the inflammation-induced rabbits by turpentine, an irritating compound (Proulx & Du Souich, 1995). In the present study, total CYP content and ANH activity in LPS-treated rabbits decreased by ≈ 30% and 40% of the control rabbits, respectively. It has been reported that total CYP content during APR was decreased by 30%–40% in pigs (Monshouwer *et al.*, 1995b, 1996), in rats (Morgan, 1989, 1993), and in horses (Auer *et al.*, 1989). The suppression of ANH activities was similar in extent with those of pigs during APR described by Monshouwer *et al.* (1996). Thus, there was a similar suppressive extent of total CYP content and/or CYP-dependent activities during APR in rabbits, pigs, rats and horses. In addition, we demonstrated that apoprotein levels of CYP1A1/1A2 and CYP2E1 also appeared to be decreased in APR-induced rabbits by Western blot analysis. Decreases in CYP1-, CYP2- and CYP3-dependent activities may lead to a reduction in the clearance of drugs, because many drugs are metabolized by these subfamilies. For example, theophylline and propranol are metabolized by CYP1A2 and benzphetamine and phenytoin are metabolized by CYP2 subfamily, and nifedipine and dextromethorphan are metabolized by CYP3A3/3A4 in humans. Although there are sex and species differences in levels of CYP-dependent activity, the activity of these enzymes is also reduced during APR in mammals.

The V_{max} and K_m of CYP-dependent enzyme catalyzed reactions during APR have never been reported in rabbits to our knowledge. We examined the changes in V_{max} and K_m values to clarify the effect of the APR on the CYP-dependent enzyme reaction. V_{max} values of CAD, AMD and ANH were found to be low compared to those in the control group. These results suggest that the amount of these enzymes was decreased during the APR, and these findings of V_{max} analysis were supported by the result of Western blot analysis. K_m values of those enzymes in group I were similar to those of the control group, suggesting that no factor which inhibited the reaction of CYP-dependent enzymes and their substrates occurred during the APR.

Antipyrine has been used widely as a probe, model drug for the assessment of hepatic oxidative metabolizing ability. The values of pharmacokinetic parameters for antipyrine have been reported in horses (Dyke *et al.*, 1998), in pigs (Monshouwer *et al.*, 1995a, 1996), in dwarf goats (Witkamp *et al.*, 1991; Offiah *et al.*, 1992), in rats (Teunissen *et al.*, 1983a; Witkamp *et al.*, 1991), in rabbits and in cattle (Witkamp *et al.*, 1991). In the present study, the Cl_{tot} for antipyrine in LPS-treated rabbits (group I) appeared to be decreased by 33% of the control rabbits. The value was almost similar to that of normal dwarf goats (Witkamp *et al.*, 1991; Offiah *et al.*, 1992). It has been reported that Cl_{tot} for antipyrine during APR decreased in pigs (Monshouwer *et al.*, 1996) and in humans (Shedlofsky *et al.*, 1994), respectively. The formation of the three main metabolites of antipyrine, 3-hydroxymethylantipyrine (HMA), 4-hydroxyantipyrine (OHA) and norantipyrine (NORA), were shown to be regulated by different types of CYP subfamily in rats (Teunissen *et al.*, 1983a). The formation of HMA, OHA and NORA were mediated by CYP1A2 and CYP2C9, CYP1A2 and CYP3A4, and CYP1A2 and CYP2C subfamilies in the human, respectively (Engel *et al.*, 1996). The relative contribution of the metabolites, HMA, OHA and NORA, to excretion in urine has been shown to be 13%–14%, 65%–70%, and 14% in normal rabbits, respectively (Witkamp *et al.*, 1991). Accordingly, Cl_{tot} for antipyrine may be influenced dominantly by the apoprotein levels of CYP1A2 and CYP3A4. In fact, we confirmed a decrease in CAD (CYP1A2) activity in LPS-treated rabbits, however, we did not determine CYP3A4-dependent activity.

It has been reported that plasma concentrations of ALB were reduced during the APR in humans (Kushner, 1982) and horses (Auer *et al.*, 1989). We also confirmed a decrease in plasma ALB concentrations by ≈ 20% of the pre-treatment level at 3 days after the LPS injection in rabbits. A decrease in ALB induces an increase in unbound fraction of a drug, which is known to alter drug disposition including apparent volume of distribution and elimination, in particular, for drugs that are highly bound to plasma ALB. In fact, Mills *et al.* (1997) have reported that the APR induced by Freund's complete adjuvant caused an ≈ 4-fold increase in unbound fraction of phenylbutazone (highly bound to plasma ALB; >98%) in horses. Alpha$_1$-acid glycoprotein (AGP) is also a plasma protein which binds with several basic drugs. AGP concentration is known to increase in response to the APR (Kushner, 1982; Van Miert, 1991), and to influence the disposition of several basic drugs (Kremer *et al.*, 1988; Tagawa *et al.*, 1994; Kinoshita *et al.*, 1995). Although, unfortunately, we have not determined the plasma AGP concentrations in the

present study, the level may increase sufficiently to influence the drug disposition in rabbits.

As mentioned above, it has been demonstrated in the present study using rabbits that hepatic microsomal CYP-dependent activities including drug metabolism are suppressed during APR, and the suppression continues for several days after the clinical findings of APR have disappeared. Also, because the alterations of parameters which indicate the APR, including total CYP contents and/or CYP-dependent activities, are very similar to those which are cited in the literature of other animals, rats (Morgan, 1989, 1993), pigs (Monshouwer *et al.*, 1996), and horses (Mills *et al.*, 1997), the suppression of CYP-dependent activities during APR may also continue for several days in the other animal species. Consequently, it may be important to establish appropriate dosage regimens after the APR.

In conclusion, the ability to oxidatively metabolize the drugs studied was supressed in incipient APR induction in rabbits, and the drug metabolizing ability was returned to normal at 7 days after APR induction. UDP-glucurosyl transferase activity, however, was not affected.

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