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Time-dependent Effects of *Klebsiella pneumoniae* Endotoxin on Hepatic Drug-metabolizing Enzyme Activity in Rats

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Abstract

The time-dependent effects of *Klebsiella pneumoniae* endotoxin on hepatic cytochrome P450-dependent drug-metabolizing capacity (cytochrome P450 and b_5 content, activity of aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase, aniline hydroxylase and benzphetamine *N*-demethylase) and on the pharmacokinetics of antipyrine have been determined in rats. Measurement of enzyme activity and antipyrine (after intravenous injection of 20 mg kg^{-1}) were performed 2, 24 and 96 h after a single intraperitoneal injection of endotoxin (1 mg kg^{-1}) and after repeated doses (once daily for 4 days). The contribution of tumour necrosis factor α (TNFα) to the endotoxin-induced changes was also examined in rats pretreated with granulocyte colony-stimulating factor (G-CSF).

The systemic clearance of antipyrine and the activity of hepatic cytochrome P450-dependent drug-metabolizing enzymes were dramatically reduced 24 h after a single injection of endotoxin, but had returned to control levels by 96 h. The magnitudes of these decreases in these measurements after repeated doses of endotoxin were similar to those seen 24 h after the single dose. The systemic clearance of antipyrine correlated significantly with cytochrome P450 content and aminopyrine *N*-demethylase activity. In histopathological experiments, moderate hypertrophy of Kupffer cells was observed, with no evidence of severe liver-tissue damage. G-CSF pretreatment suppressed the increased plasma concentrations of TNFα produced 2 h after single endotoxin injection, but did not eliminate the endotoxin-induced decrease in the systemic clearance of antipyrine, suggesting that TNFα is not the sole component responsible for the reduction of cytochrome P450-mediated drug-metabolizing enzyme activity.

These results provide evidence that a single intraperitoneal injection of 1·0 mg kg^{-1} *K. pneumoniae* endotoxin in rats reduces hepatic P450 and b_5 levels, and reduces the activity of various cytochrome P450-mediated drug-metabolizing enzymes without causing severe liver-tissue damage. This suggests that the effect of endotoxin on hepatic cytochrome P450-mediated drug-metabolizing isozymes is non-selective.

Endotoxin, a component of the cell wall of Gram-negative bacteria, has a variety of biological and immunological activity. Endotoxin-induced physiological changes in several organs modify the pharmacokinetic behaviour of drugs (Lodefoged 1977; Bergeron & Bergeron 1986; Ganzinger et al 1986; Auclair et al 1990; Tardif et al 1990). In a series of studies we have found that *Klebsiella pneumoniae* endotoxin dramatically reduces renal excretion of organic anionic drugs by reducing glomerular filtration and tubular secretion (Nadai et al 1993a, b, c, 1996, Hasegawa et al 1994). More recent studies in our laboratory have also reported that *K. pneumoniae* endotoxin dramatically reduces biliary excretion of the organic anionic drug cefoperazone, which is actively secreted into the bile (Haghgoo et al 1995). Several investigators have demonstrated that endotoxin damages the liver as a result of the uptake of endotoxin both by hepatocytes and by Kupffer cells (Praaning-van Dalen et al 1981; Ruiter et al 1981; Freudenberg et al 1982;

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Van Bossuyt et al 1988) and that it reduces both the content and the activity of hepatic cytochrome P450-mediated drug-metabolizing enzymes (Gorodischer et al 1976; Yaffe & Sonawane 1978; Williams et al 1980). Thus, endotoxin might delay the elimination of drugs which are almost completely metabolized in the liver by depression of hepatic cytochrome P450 isozymes.

It has been reported that the activity of hepatic drug-metabolizing enzymes in rats decreases 2 h after administration of endotoxin (Gorodischer et al 1976) and that the activity of the hepatic type O form of xanthine oxidase in mice was increased by treatment with interleukin-1 (IL-1), a cytokine released by endotoxin stimulation (Cantoni et al 1995). We have previously demonstrated that 2-h pretreatment with endotoxin has little or no effect on the metabolism of theophylline catalysed by the cytochrome P450 monooxygenase system in rats (Wang et al 1993). This discrepancy might be explained by evidence showing that the contribution of the liver to theophylline metabolism in rats is relatively low (approximately 40% of theophylline administered; Nadai et al 1990; Wang et al 1993) and that endotoxin has a time-dependent effect on drug pharmacokinetics (Nadai et al 1995).

There is little information currently available on the time-dependent effects of endotoxin on the activity of hepatic drug-metabolizing enzymes in animals and man. This study was thus designed to determine whether *K. pneumoniae* endotoxin time-dependently reduces the activity of hepatic cytochrome P450-mediated drug-metabolizing enzymes, by using antipyrine as a model substrate in rats. Antipyrine is widely used as a probe to evaluate the capacity of drug metabolism, because it is almost completely metabolized by the hepatic cytochrome P450 isozymes in rats (Inaba et al 1980). It is also well known that the protein-binding potency of antipyrine is negligible and its elimination is independent of hepatic blood flow, which is changed by endotoxin. The effect of endotoxin on cytochrome P450 and cytochrome b_5 content and on the activity of enzymes for four kinds of substrate was investigated. Most recently, we found that pretreatment with granulocyte colony-stimulating factor (G-CSF) enhances the endotoxin-induced decrease in the biliary excretion of organic anion drugs (unpublished data). Therefore, the effect of G-CSF on hepatic cytochrome P-450-dependent drug metabolism was also investigated.

Materials and Methods

Chemicals

Antipyrine and phenacetin were purchased from Sigma (St Louis, MO). Endotoxin was isolated from a culture supernatant of *Klebsiella pneumoniae* LEN-1 (O3 : K1$^-$) (Hasegawa et al 1983, 1985), which is a decapsulated mutant strain derived from the *K. pneumoniae* strain of Kasuya (O3 : K1) as previously described (Ohta et al 1981). Recombinant granulocyte colony-stimulating factor (G-CSF) from man was kindly donated by Chugai (Tokyo, Japan) in the form of a commercial preparation kit for injection (Neutrogin). All other chemicals used were obtained commercially and were used without further purification. Endotoxin, antipyrine and G-CSF were dissolved in isotonic saline.

Animal experiments

Male Wistar rats, 8–9 weeks (Japan SLC, Hamamatsu, Japan), were used in all experiments. Rats were allowed free access to food and water during experiments. In the single-dose experiments, rats received a bolus intravenous injection of antipyrine (20 mg kg^{-1}) 2, 24 and 96 h after a single intraperitoneal injection of endotoxin at a dose of 1·0 mg kg^{-1}. In the repeated dose experiments, rats were given intraperitoneal injections of isotonic saline or endotoxin (1·0 mg kg^{-1}) once daily for 4 days (0900 h). Antipyrine pharmacokinetics were determined on the 5th day (0900 h). To study the effects of G-CSF pretreatment on the activity of hepatic drug-metabolizing enzymes, rats received G-CSF subcutaneously (12 μg kg^{-1} daily for 4 days) and received a final dose of G-CSF (50 μg kg^{-1}) 1 h before endotoxin injection. Antipyrine (20 mg kg^{-1}) was administered intravenously 24 h after endotoxin injection and the pharmacokinetics of antipyrine were determined.

One day before these experiments rats were anaesthetized with sodium pentobarbital (25 mg kg^{-1}) and the right jugular vein was cannulated with polyethylene tubing for administration of drugs and for blood sampling. Blood samples (approx. 0·25 mL) were collected 30, 60, 90, 120, 180, 240 and 300 min after antipyrine administration. Plasma samples were immediately obtained by centrifugation at 6000 *g* for 5 min and were stored at −40°C until analysis.

Histopathological experiments

Rats were killed by exsanguination under light anaesthesia with sodium pentobarbital (25 mg kg^{-1}) 24 h after an intravenous administration of saline or endotoxin (1 mg kg^{-1}) and the livers were immediately removed. For light microscopy small pieces of liver tissue were fixed in 10% formaldehyde in neutral phosphate-buffered saline. Fixed tissue specimens were dehydrated by graded ethanol treatment and were routinely embedded in paraffin wax for light microscopy. Embedded

sections (2–4 μm) were stained with haematoxylin and eosin (H & E) and periodic acid–Schiff reagent. For electron microscopy small pieces of liver tissue were fixed in phosphate-buffered 2% glutaraldehyde, further fixed in 1% osmium tetroxide, and embedded in epoxy resin. The ultra-thin sections were stained with uranyl acetate and lead citrate and then examined with a JEM 100S electron microscope.

The embedded sections (2–4 μm) were reacted with a mouse antibody against rat monocytes and macrophages (ED-1, Serotec, 1 : 100) and binding sites were visualized by use of the labelled streptavidine biotin complex method (LSAB, DAKO kit) for immunohistochemical staining of ED-1. Histopathological examination was performed by veterinary pathologists.

Biochemical determinations

Rats were killed by exsanguination under light anaesthesia with sodium pentobarbital (25 mg kg^{-1}) 2, 24 and 96 h after single doses and 24 h after repeated doses of endotoxin (1 mg kg^{-1}) and livers were immediately excised and weighed. The left lobe was removed and perfused with ice-cold 1·15% KCl solution for microsome preparation; the other lobes of the liver were fixed in 10% neutral buffered formalin. The perfused liver lobes were homogenized in 250 mM sucrose–10 mM Tris-HCl (pH 7·4) and centrifuged at 12 000 *g* for 10 min. The supernatant fraction was ultracentrifuged at 105 000 *g* for 60 min and the microsomal pellet was resuspended in ice-cold 1·15% KCl–10 mM Tris-HCl (pH 7·4).

Cytochrome P450 and cytochrome b_5 content were determined by the methods of Estabrook & Werringloer (1978) and Omura & Sato (1964). Concentrations of microsomal proteins were determined by the Pyrogallol-red method (Fujita et al 1983). Aniline hydroxylase and *p*-nitroanisole–*O*-demethylase activity were measured by the methods of Imai et al (1966) and Kamataki et al (1979), respectively. *N*-Demethylase and benzphetamine *N*-demethylase activity were measured by the method of Nash (1953).

Blood chemical analysis for L-alanine aminotransferase, L-aspartate aminotransferase and alkaline phosphatase activity in plasma obtained from rats 24 h after a single treatment with or without endotoxin was performed by means of an Hitachi (Japan) 736-40 automatic analyser. Peripheral blood-cell number was measured by means of a Toa Medical Electronics (Japan) Sysmex E-3000 fully automatic haematology analyser.

TNFα concentrations in plasma 2 h after intraperitoneal injection of saline or endotoxin (1 mg kg^{-1}) in rats pretreated with or without G-CSF were measured by means of the Biotrak mouse TNFα ELISA system (Amersham International, UK). Rats received subcutaneous G-CSF (12 mg kg^{-1} daily) for 4 days followed by a final dose of G-CSF (50 mg kg^{-1}) 1 h before endotoxin injection.

Drug analysis

Plasma antipyrine concentrations were measured by high-performance liquid chromatography (HPLC), using a slight modification of a previously described method (Pilsgaard & Poulsen 1984). HPLC was performed with a Shimadzu (Kyoto, Japan) LC-6A system consisting of an LC-6A liquid pump, an SPD-6A UV spectrophotometric detector (operated at 254 nm), an SIL-6A autoinjector and an OTC-6A column oven (40°C). Compounds were separated on a Nacalai Tesque (Kyoto, Japan) 4·6 mm × 150 mm Cosmosil $5C_{18}$ column; the mobile phase was 30 mM isotonic phosphate buffer (pH 5·0)–acetonitrile, 75 : 25 (v/v) and the flow rate was 1·5 mL min^{-1}.

Phenacetin was used as internal standard. Standard curves for antipyrine were measured over the range 0·5–50 μg mL^{-1} and proved to be linear (correlation coefficient 0·999). The intra- and interday coefficient of variation for the desired concentrations (5 and 20 μg mL^{-1}) was < 6%. The quantitative limit of detection of antipyrine by this method was 0·2 μg mL^{-1}.

Data analysis

Plasma concentration–time data for antipyrine in each rat were individually analysed by non-compartmental methods using the non-linear least-squares regression program MULTI (Yamaoka et al 1981). The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal method up to the last measured concentration in plasma and were extrapolated to infinity. Systemic clearance (CL) was calculated as the dose divided by the AUC. The steady-state volume of distribution was calculated as CL × MRT, where MRT represents the mean residence time and was calculated as AUMC/AUC.

Statistical analysis

Results are expressed as mean ± s.e. for the indicated number of experiments. Statistical comparisons between the groups were assessed by one-way analysis of variance. P values < 0·05 were accepted as evidence of a statistically significant difference.

Results and Discussion

There is evidence that the turnover time of P450 in rats is 2 to 4 days (Arias & DeLeon 1967; Levin & Kuntzman 1969). More recently, Sewer et al (1996) have demonstrated that mRNA of hepatic cytochrome P450 isozymes 2C11, 2E1, and 3A2 was decreased 6 to 48 h after endotoxin injection in rats. The current study, therefore, examined the time-dependent effects of endotoxin on hepatic cytochrome P450-dependent drug-metabolizing enzymes.

The time-dependent effects of *K. pneumoniae* endotoxin on the activities of hepatic P-450-dependent drug-metabolizing enzymes were evaluated in rats using antipyrine as a probe drug, because antipyrine is completely metabolized by at least six hepatic cytochrome P450 isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18 and CYP3A4) (Engel et al 1996). Figure 1 shows the mean semilogarithmic plots of plasma antipyrine concentration–time data after a bolus injection of 20 mg kg^{-1} to rats pretreated 2, 24 or 96 h earlier with endotoxin. Data obtained 24 h after repeated doses of endotoxin are also shown in Figure 1. Compared with control rats no changes in the plasma concentration–time curves for antipyrine were observed for rats pretreated with endotoxin 2 and 96 h before antipyrine administration. However, disappearance of antipyrine from plasma was markedly delayed when the drug was administered 24 h after either single or repeated doses of endotoxin. Endotoxin-induced changes in the systemic clearance of antipyrine are illustrated in Figure 2. As this figure shows, the systemic clearance of antipyrine was significantly reduced in rats 24 h after a single administration of endotoxin.

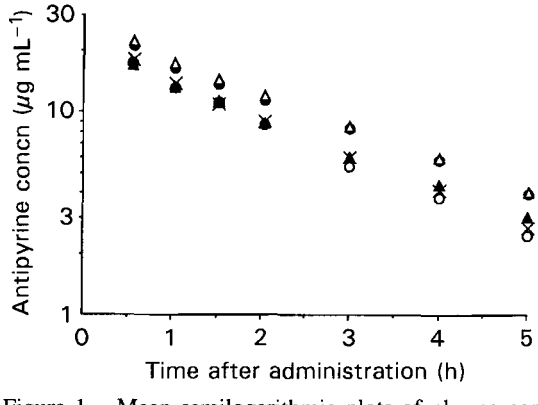

Figure 1. Mean semilogarithmic plots of plasma concentration–time data for antipyrine in untreated rats (○) and in rats pretreated with endotoxin at a dose of 1 mg kg^{-1} (▲, △, ×, ●). Antipyrine (20 mg kg^{-1}) was administered intravenously 2 (▲), 24 (△) and 96 h (×) after a single administration of endotoxin and 24 h after its repeated administration (●). Each symbol represents the mean of results from six to nine rats.

Repeated doses of endotoxin were found to elicit a similar reduction in the systemic clearance of antipyrine ($0{\cdot}318 \pm 0{\cdot}042$ L h^{-1} kg^{-1}), although single or repeated administration of endotoxin had no effect on the volume of distribution of antipyrine (data not shown).

The time-dependent effects of endotoxin on the activity of hepatic drug-metabolizing enzymes are shown in Figures 3–5. Hepatic cytochrome P450 and b_5 levels were reduced to 50% (0·37 nmol (mg protein)$^{-1}$) and 70% (0·27 nmol (mg protein)$^{-1}$) of control levels, respectively, 24 h after single administration of endotoxin to rats; both values returned to control levels within 96 h (Figure 3). Repeated treatment with endotoxin induced reductions to 70% (0·51 nmol (mg protein)$^{-1}$) and 78% (0·31 nmol (mg protein)$^{-1}$) of control levels, respectively. As can be seen in Figures 4 and 5, the activity of four kinds of hepatic drug-metabolizing enzyme (aminopyrine *N*-demethylase (CYP3A4) (Anderson et al 1997), aniline hydroxylase (CYP2E1) (Monostory & Vereczkey 1995), benzphetamine *N*-demethylase (CYP2B) (Ahmed et al 1995) and *p*-nitroanisole *O*-demethylase (mixed) (Bauer et al 1994)) were significantly reduced by 50–70% 24 h after administration; this decrease was similar to that observed for cytochrome P450 and b_5 levels. Aminopyrine *N*-demethylase and benzphetamine *N*-demethylase activity did not return completely to control levels. Enzyme activity was also reduced 24 h after repeated endotoxin treatment, perhaps more than after the single

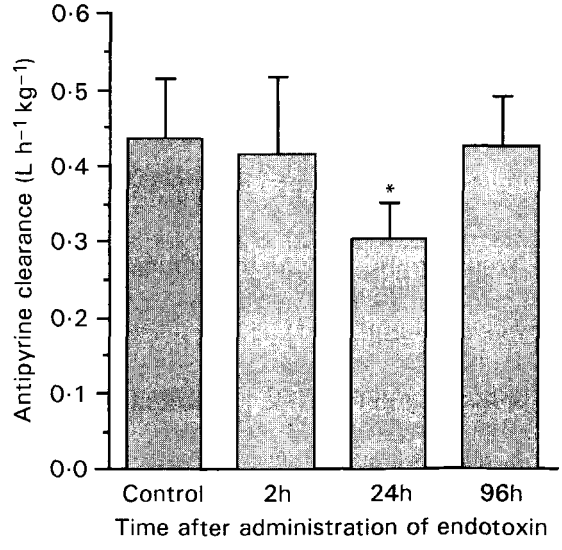

Figure 2. Time-dependent changes in the systemic clearance of antipyrine after administration of endotoxin. Antipyrine was administered 2, 24 and 96 h after a single injection of endotoxin. Each column represents the mean ± s.e.m. of results from six to nine rats. $*P < 0{\cdot}05$, significantly different from control result.

treatment, especially for benzphetamine *N*-demethylase, the activity of which was reduced by approximately 70% after repeated doses in comparison with a reduction of approximately 55% after the single dose. If endotoxin inhibits the synthesis of hepatic cytochrome P450, it could be hypothesized that repeated administration would result in greater depression of hepatic drug-metabolizing capability than administration of a single dose (Arias & DeLeon 1967; Levin & Kuntzman 1969). The results presented here thus suggest that endotoxin does not produce its effect by inhibiting hepatic cytochrome P450 synthesis. The findings that repeated endotoxin treatment did not result in more profound depression of hepatic drug-metabolizing enzyme activity might be explained by induction of tolerance to endotoxin or by inflammatory stimulation (Williams 1985; Shedlofsky et al 1994). Although the precise mechanism responsible for time-dependent differences in depression of substrate metabolism by endotoxin has not been addressed by these experiments, we propose that they might result from differential effects of endotoxin on the expression and activity of various cytochrome P450 isozymes in the liver.

Changes in hepatic drug-metabolizing capacity could lead to changes in the systemic clearance of antipyrine. We therefore evaluated possible relationships between the systemic clearance of antipyrine and either cytochrome P450 level or aminopyrine *N*-demethylase activity in all rat groups. As shown in Figure 6, the systemic clearance of antipyrine correlated significantly both with P450 level and with aminopyrine *N*-demethylase activity ($r = 0.971$ and 0.928, respectively). Statistically significant correlations were also observed between the systemic clearance of antipyrine and both the concentration of cytochrome b_5 and the activity of other hepatic microsomal enzymes (data not shown). These results confirm that changes in the systemic clearance of antipyrine reflect changes in the activity of hepatic P450-dependent drug-metabolizing enzymes.

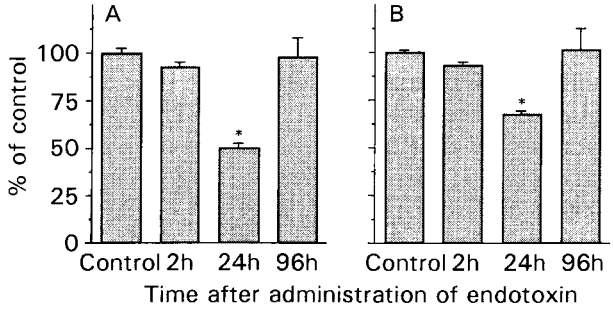

Figure 3. Time-dependent changes in cytochrome P450 (A) and b_5 (B) content 2, 24 and 96 h after a single injection of endotoxin. Each content is represented as the percentage of that of the control. The mean control content of cytochrome P450 and b_5 was 0.7430 and 0.404 nmol (mg protein)$^{-1}$, respectively. Each column represents the mean ± s.e.m. of results from five rats. *$P < 0.05$, significantly different from control result.

It was previously thought that endotoxin-induced depression of hepatic cytochrome P450 was caused by enhancement of the degradation of cytochrome P450 haem proteins (Bissell & Hammaker 1976a, b). Several publications have recently suggested that endotoxin-induced depression of hepatic P450-mediated drug metabolism is probably caused by suppression of protein translation and mRNA transcription for cytochrome P450 isozymes by certain inflammatory cytokines, such as tumour necrosis factor (TNF), interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6) (Stanley et al 1988; Bertini et al 1989, 1992; Morgan 1989; Ishikawa et al 1991; Cantoni et al 1995). The effects of the inflammatory response on hepatic cytochrome P450-mediated drug-metabolizing enzyme activity are currently being extensively studied.

It is well known that endotoxin induces increased levels of cytokines, such as TNF, IL-1 and IL-6 and the resulting elevation of these cytokines might play an important role in endotoxin-induced changes in the activity of hepatic drug-metabolizing enzymes. Shedlofsky et al (1994) have reported that the systemic clearance of antipyrine correlates significantly with endotoxin-induced serum concentrations of cytokines (TNF and IL-6), indicating that the inflammatory response to endotoxin plays an important role in reduced hepatic cytochrome P450-dependent drug metabolism. These observations also suggest the possibility that hepatic P450-mediated drug-metabolizing enzyme activity can be predicted by measuring the systemic clearance of antipyrine or the peak serum concentration of TNFα or IL-6. Duan et al (1988) have also reported that the disappearance of aminopyrine and diazepam from the body is delayed in rats pretreated with TNFα. Ghezzi et al (1986a, b) have demonstrated that TNFα depresses cytochrome P450 levels in-vivo, but not in-vitro, in cultured hepatocytes. They further demonstrated that depression of hepatic cytochrome P450 levels by endotoxin or TNFα is mediated by IL-1. The current study, therefore, investigated the role of TNFα in endotoxin-induced decreases in hepatic drug-metabolizing enzyme activity and antipyrine clearance. Plasma TNFα concentrations 2 h after administration of saline, endotoxin, G-CSF alone or G-CSF plus endotoxin (G-CSF–endotoxin) are represented in Figure 7. G-CSF alone or endotoxin markedly increased the level of TNFα (57.4 ± 4.7 and

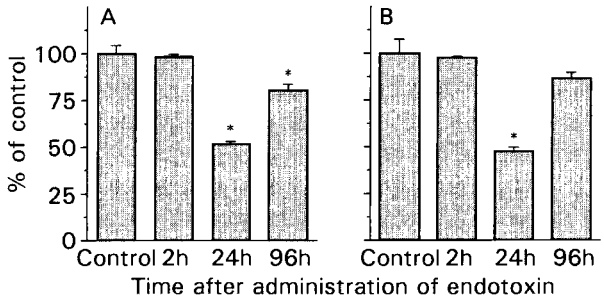

Figure 4. Time-dependent changes in microsomal activity of aminopyrine *N*-demethylase (A) and aniline hydroxylase (B) 2, 24 and 96 h after a single injection of endotoxin. Each activity is represented as the percentage of that of the control (131·8 and 18·8 nmol/30 min (mg protein)$^{-1}$, respectively, for aminopyrine *N*-demethylase and aniline hydroxylase). Each column represents the mean ± s.e.m. of results from five rats. *$P < 0.05$, significantly different from control result.

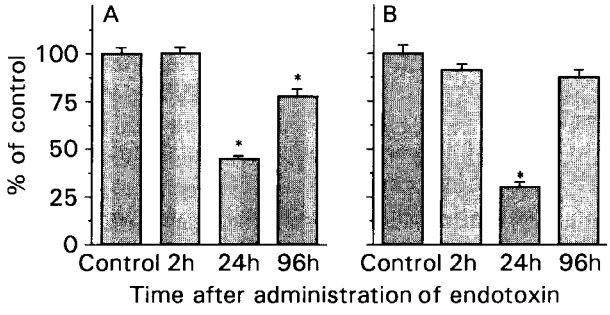

Figure 5. Time-dependent changes in microsomal activity of benzphetamine–*N*-demethylase (A) and *p*-nitroanisole *O*-demethylase (B) 2, 24 and 96 h after a single injection of endotoxin. Each activity is represented as the percentage of that of the control (184·0 and 18·2 nmol/30 min (mg protein)$^{-1}$, respectively, for benzphetamine–*N*-demethylase and *p*-nitroanisole *O*-demethylase). Each column represents the mean ± s.e.m. of results from five rats. *$P < 0.05$, significantly different from control result.

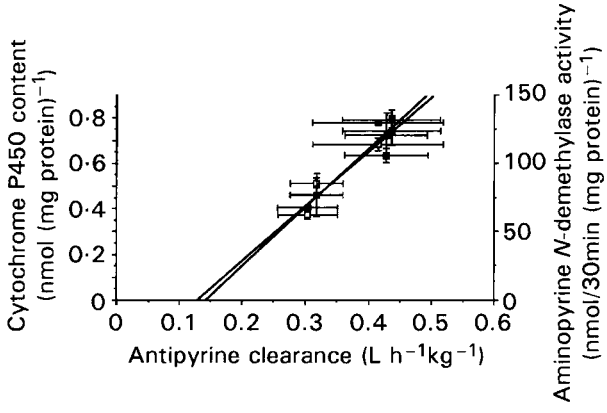

Figure 6. Relationships between the systemic clearance of antipyrine and cytochrome P450 content (□) or aminopyrine *N*-demethylase activity (■). Each symbol represents the mean ± s.e.m. of results from five rats. The correlation coefficient was 0·971 for cytochrome P450 content and 0·928 for aminopyrine *N*-demethylase activity.

45·9 ± 7·6 pg mL^{-1}, respectively), whereas G-CSF pretreatment markedly inhibited the production of TNFα induced by endotoxin (20·8 ± 3·9 pg mL^{-1}) to a level not different from control (18·2 ± 3·0 pg mL^{-1}). These results are consistent with those previously reported by Santucci et al (1995), who demonstrated that G-CSF prevents TNFα release induced by endotoxin. On the basis of these results, we examined whether G-CSF can eliminate the reduction in the systemic clearance of antipyrine induced by a single 24 h pretreatment with endotoxin (1 mg kg^{-1}). G-CSF alone had no effects on the plasma concentration–time curves or systemic clearance of antipyrine (data not shown). These results suggest that G-CSF and the TNFα produced by G-CSF pretreatment have no effect on hepatic drug-metabolizing enzyme activity. In addition, no significant differences between the systemic clearance of antipyrine were observed for endotoxin-treated and G-CSF and endotoxin-treated rats (data not shown), indicating that G-CSF pretreatment cannot block the decrease in the systemic clearance of antipyrine induced by endotoxin injection. Rather, these data support the hypothesis that the endotoxin-induced decrease in hepatic cytochrome P450-mediated drug metabolism is a result of endotoxin-induced elevations of IL-1 or IL-2 (Shedlofsky et al 1987; Bertini et al 1989; Cantoni et al 1995). We might surmise that the suppression of hepatic cytochrome P450-dependent drug-metabolizing activity by endotoxin is caused by the synergistic action of more than one cytokine.

In this study obvious biochemical and haematological changes were observed 24 h after endotoxin treatment (Table 1). Biochemical effects included significantly reduced alkaline phosphatase concentrations and 1·5-fold increased plasma L-alanine aminotransferase levels. Endotoxin-induced haematological effects include minor but significant increases in red blood cell counts and haematocrit and markedly reduced platelet counts (approximately 40% of control levels).

In the histopathological experiments, moderate hypertrophy of Kupffer cells was observed in all rats 24 h after the single dose of endotoxin (Figure 8) and in 3 of 5 rats 24 h after the repeated treatment, suggesting the absence of severe liver-tissue damage. Previous studies in our laboratory have found that intravenous injection of endotoxin (250 μg kg^{-1}) elicited an immediate reduction in renal function without inducing any histopathological changes in the kidneys; the reduced renal functions returned to normal within 24 h (Nadai et al 1993c, 1995). It is likely that the endotoxin-induced reduction of hepatic drug-metabolizing

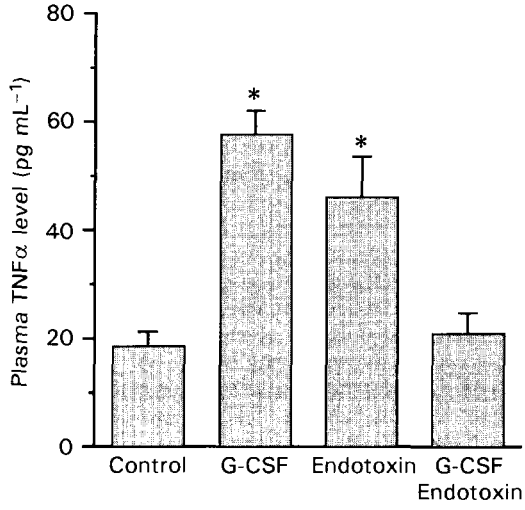

Figure 7. The role of granulocyte colony-stimulating factor (G-CSF) in the production of tumour necrosis factor-α (TNFα) induced by endotoxin. Rats received subcutaneous G-CSF (12 mg kg^{-1} daily) for 4 days followed by a final dose of G-CSF (50 mg kg^{-1}) 1 h before endotoxin injection (1 mg kg^{-1}). Plasma TNFα levels were measured 2 h after administration of endotoxin. Each column represents the mean ± s.e.m. of results from five rats. $*P < 0.05$, significantly different from control result.

capability is a slower than the reduction of renal functions. On the basis of histopathological examination, it might be suggested that changes in the activity of hepatic cytochrome P450-mediated drug-metabolizing enzymes are not directly related to the alteration in hepatic function induced by endotoxin or cytokines.

In conclusion, we have provided evidence that a single intraperitoneal injection of *K. pneumoniae* endotoxin (1·0 mg kg^{-1}) in rats reduces both hepatic P450 and b_5 levels and the activity of some cytochrome P450-mediated drug-metabolizing enzymes without causing severe liver-tissue

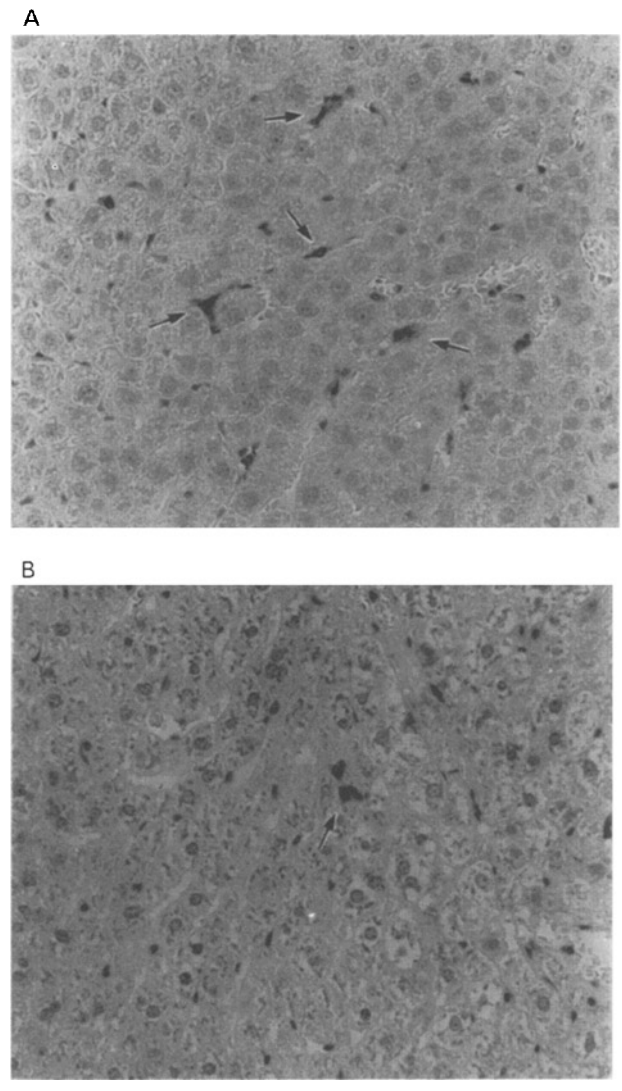

Figure 8. Light micrographs of liver stained with anti-rat ED1 antibody. Liver was isolated from rats pretreated with endotoxin 24 h earlier (A) and from untreated rats (B). Arrows show Kupffer cells. Magnification, × 210.

Table 1. Biochemical and haematological parameters for untreated rats and for rats pretreated with endotoxin 24 h earlier.

Parameter	Treatment	
	Without	With
Alkaline phosphatase (Iunits L^{-1})	883·0 ± 35·4	614·6 ± 9·9*
L-Alanine aminotransferase (Iunits L^{-1})	44·2 ± 4·3	69·6 ± 12·1
L-Aspartate aminotransferase (Iunits L^{-1})	75·0 ± 4·9	114·8 ± 9·6*
Total protein (g dL^{-1})	5·62 ± 0·07	5·74 ± 0·10
Total cholesterol (mg dL^{-1})	45·2 ± 1·9	56·6 ± 5·1
Red blood cells ($\times 10^4$ μL^{-1})	860·2 ± 3·7	914·6 ± 10·4*
Haematocrit (%)	44·68 ± 0·21	46·30 ± 0·51*
Haemoglobin (g dL^{-1})	14·90 ± 0·16	15·56 ± 0·20*
White blood cells ($\times 10^3$ μL^{-1})	4·78 ± 0·15	5·38 ± 0·32
Platelets ($\times 10^3$ μL^{-1})	77·04 ± 3·21	28·16 ± 4·37*

Data means ± s.e. (n = 5). $*P < 0.05$, significantly different from control result.

damage. The maximum decrease occurs 24 h after endotoxin administration with return to control levels by 96 h. This study suggests that the effect of endotoxin on hepatic cytochrome P450-mediated drug-metabolizing isozymes is non-selective. The effects of endotoxin observed in this study are in partial agreement with those reported previously (Morgan 1989; Shedlofsky et al 1994).

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