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Effect of pioglitazone on endotoxin-induced decreases in hepatic drug-metabolizing enzyme activity and expression of CYP3A2 and CYP2C11

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Abstract

It has been reported that peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands ameliorate the expression of inducible nitric oxide synthase (iNOS) by endotoxin. In the present study, we investigated the effect of pioglitazone, a potent PPAR-γ ligand, on the endotoxin-induced reduction of hepatic drug-metabolizing enzyme activity and on the down-regulation of the expression of hepatic cytochrome P450 (CYP) 3A2 and CYP2C11 proteins in rats. Endotoxin (1 mg/kg) significantly decreased hepatic drug-metabolizing enzyme activity in vivo, as represented by the systemic clearance of antipyrine and protein levels of CYP3A2 and CYP2C11 24 h after intraperitoneal injection. Pretreatment with pioglitazone (10 mg/kg, 4 times at 10-min intervals) significantly protected the endotoxin-induced decreases in the systemic clearance of antipyrine and protein levels of CYP3A2, but not CYP2C11, with no biochemical and histopathological changes in the liver. Pioglitazone alone had no effect on the systemic clearance of antipyrine and protein levels of CYP3A2 or CYP2C11. Pioglitazone significantly protected endotoxin-induced overexpression of iNOS in the liver, but not the overproduction of nitric oxide (NO) in plasma. It is unlikely that the protective effect of pioglitazone against endotoxin-induced decreases in the hepatic drug-metabolizing enzyme activity and protein levels of CYP3A2 in the liver is due to the inhibition of the overproduction of NO.

Keywords: Endotoxin; CYP3A2; CYP2C11; Pioglitazone; PPAR-γ; Antipyrine clearance; (Rat)

1. Introduction

Endotoxin, an active component of the cell wall of Gram-negative bacteria, is known to induce damage to numerous organs, including the liver. The liver has crucial functions for the detoxification of endogenous and exogenous compounds, which are converted to more hydrophilic compounds by cytochrome P450 (CYP) and/or conjugating enzymes. A number of researchers, including those in our group, have demonstrated that endotoxin reduces CYP-dependent drug metabolism (Chen et al., 1992; Kitaichi et al., 1999; Morgan, 1993; Nadai et al., 1998a,b; Sewer et al., 1996). Endotoxin has also been found to increase the expression of inducible nitric oxide synthase (iNOS) (Lee et al., 2003), and up-regulate various inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-6 and interferons (Cassatella et al., 1993; Crawford et al., 1997; Evans et al., 1993), although endotoxin is reported to have no effect on hepatocytes, which are the primary source of these inflammatory cytokines (Sauer et al., 1996).

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There are a number of papers suggesting that some cytokines reduce the activity of various CYP isoforms (Carlson and Billings, 1996; Morgan et al., 1994; Sewer and Morgan, 1997). For example, TNF-α is shown to decrease the activity of CYP1A, CYP2B, CYP2C, CYP2E and CYP3A subfamilies (Chen et al., 1992; Monshouwer et al., 1996; Nadin et al., 1995; Pous et al., 1990; Sewer and Morgan, 1997). Sewer and Morgan (1998) reported that endotoxin down-regulates the expression of hepatic CYP2C11, CYP2E1 and CYP3A2 proteins, but the down-regulation is independent of nitric oxide (NO), which is mainly produced by inducible NO synthase (iNOS). On the other hand, reports have suggested that the overproduction of NO induces the reduction of CYP-dependent drug-metabolizing enzyme activity (Minamiyama et al., 1998; Takemura et al., 1999). We previously reported that *K. pneumoniae* endotoxin decreases the systemic clearance of antipyrine and hepatic CYP-dependent drug-metabolizing enzyme activity with no histopathological changes in the liver (Nadai et al., 1998a,b). Further in vivo studies revealed that the endotoxin-induced decrease in the hepatic CYP-dependent drug-metabolizing enzyme activity is due in part to the overproduction of NO (Kitaichi et al., 1999). However, less is known about the precise mechanism responsible for endotoxin-induced decreases in the expression and function of CYP isoforms.

It is known that pioglitazone, a thiazolidinedione derivative, is used for the treatment of type II diabetic patients and binds to peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the PPAR subfamily of nuclear receptors with high affinity (Lehmann et al., 1995). Its pharmacological effects might be mediated via interaction with PPAR-γ (Willson et al., 1996). PPAR-γ is expressed in various inflammatory cells such as neutrophils (Greene et al., 1995), macrophages (Jiang et al., 1998; Ricote et al., 1998) and vascular smooth muscle cells (Law et al., 2000). It was reported that PPAR-γ activation suppresses inflammatory responses such as activation of the transcription factors activator protein 1 and nuclear factor κB (NF-κB), that bind to the promoter region of both iNOS and TNF-α genes (Jiang et al., 1998; Ricote et al., 1998). Moreover, there are many reports regarding anti-inflammatory effects due to PPAR-γ activation. In vitro experiments have shown that PPAR-γ ligands, including pioglitazone, ameliorate the expression of iNOS, which increases in various cell types, such as mesangial cells, Kupffer cells and macrophages, after exposure to immunological stimuli including endotoxin (Reilly et al., 2001; Uchimura et al., 2001). Ricote et al. (1998) reported that PPAR-γ ligands inhibit the expression of TNF-α, interleukin-6 and interleukin-1β. It is possible that pioglitazone protects against endotoxin-induced decreases in the hepatic CYP-dependent drug-metabolizing enzyme activity.

The purpose of the present study was to investigate whether pioglitazone can prevent endotoxin-induced decreases in the hepatic drug-metabolizing enzyme activity and in the expression of CYP3A2 and CYP2C11 in the liver in vivo, since CYP3A2 and CYP2C11 are major CYP subtypes in rats (Souèek and Gut, 1992) and are sensitive to several cytokines (Morgan, 1997).

2. Methods

2.1. Chemicals

Endotoxin was prepared from a clinically isolated *Klebsiella pneumoniae* LEN-1 (O3:K1$^-$), which was identical to that used in previous studies (Ando et al., 2001; Kitaichi et al., 1999; Nadai et al., 1998a,b). Pioglitazone was kindly donated by Takeda Chemical (Osaka, Japan). Antipyrine and phenacetin (an internal standard) were purchased from Sigma (St. Louis, MO, USA). All other reagents were commercially available and of analytical grade. Pioglitazone was dissolved in dimethyl sulfoxide at a final concentration of 5% in olive oil and was suspended in olive oil at a concentration of 10 mg/ml.

2.2. Animals

Eight-week-old male Wistar rats (Japan SLC, Hamamatsu, Japan) were used in all experiments. The rats were housed under controlled environmental conditions (temperature of 22–24 °C and humidity of 55±5%) with a commercial food diet and water freely available to animals for at least 3 days before the experiment and surgery. The procedures involving animals and their care conformed to the international guidelines, Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) and Guiding Principles for the Care and Use of Laboratory Animals of Nagoya University, Japan.

2.3. In vivo experiments

One day before the start of the experiments, rats were anesthetized with sodium pentobarbital (25 mg/kg of body weight), and the right jugular vein was cannulated with polyethylene tubes (Natsume PE45, Tokyo, Japan) for drug administration and blood sampling. The peritoneal cavity was also cannulated with polyethylene tubes for drug administration. The rats were intraperitoneally administered pioglitazone (10 mg/kg of body weight) at designated intervals of 60, 50, 40 and 30 min before intraperitoneal injection of endotoxin (1 mg/kg of body weight). The injection volume for pioglitazone and endotoxin was 0.1 ml/100 g and 0.2 ml/100 g, respectively. Control animals received an equivalent volume of sterile saline and olive oil at designated intervals in place of endotoxin and pioglitazone, respectively. The rats were killed by exsanguination from the abdominal aorta under light anesthesia. The liver was removed after perfusion with cold saline at 6, 12 and 24 h after injection of endotoxin.

Twenty-four hours after injection of endotoxin or saline, the antipyrine clearance experiments were done. Antipyrine (20 mg/kg body weight) was administered intravenously to endotoxin-, endotoxin plus pioglitazone-, pioglitazone alone-treated or saline-treated (control) rats. Blood samples were collected at designated intervals of 30, 60, 90, 120, 180, 240 and 300 min after antipyrine administration. Plasma samples were immediately obtained by centrifugation at 1200×*g* for 5 min at 4 °C and were stored at −40 °C until analyzed.

2.4. Histopathological examinations

The rats were lightly anesthetized with sodium pentobarbital (25 mg/kg) and killed by exsanguination 24 h after injection of endotoxin or saline. The liver was immediately removed and small pieces of liver were fixed in 20% formaldehyde in neutral phosphate-buffered saline and routinely embedded in paraffin wax for light microscopy. Paraffin sections were treated with hematoxylin and eosin stain. Veterinary pathologists performed the histopathological examinations according to a method reported previously (Zhao et al., 2002).

2.5. Biochemical tests

The concentrations of albumin and total protein in plasma were determined by using the well-known bromcresol green and biuret reactions, respectively. The concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol and cholinesterase in the plasma were measured with commercial kits (Wako, Tokyo, Japan).

2.6. Drug assay

The concentrations of antipyrine in plasma were determined by high-performance liquid chromatography (HPLC) according to a method described previously (Kitaichi et al., 1999). The apparatus used for HPLC was a Shimadzu LC-10A system (Kyoto, Japan) consisting of an LC-10A liquid pump and an auto injector SIL-10Advp, and equipped with a UV–VIS detector (SPD-10 AV) set at a wavelength of 254 nm. The assay conditions were as follows: column, a LiChroCART (Merck, Tokyo, Japan); mobile phase, 30% (v/v) methanol in purified water (18 MΩ); temperature, 40 °C; flow rate, 1.0 ml/min.

2.7. Assay of nitric oxide

The concentrations of NOx (NO_2^- and NO_3^-) in plasma were measured with a commercial kit (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical, Ann Arbor, MI). Briefly, the plasma samples obtained from in vivo experiments were ultrafiltered (molecular cut-off of 10,000) at 6000×*g* for 60 min at 4 °C. The ultrafiltrate was allowed to incubate for 3 h with nitrate reductase and its cofactor and to react with Griess reagents for 20 min. Absorbance was measured at 540 nm with a microplate reader (Molecular Devices, Crawley, UK) and converted to NOx concentrations using a nitrate standard curve. Recovery of nitrite in this assay was over 95%.

2.8. Western blot analysis

The preparation of microsomes was described elsewhere (Omura and Sato, 1964). Briefly, liver (approximately 1 g) was homogenized at 4 °C with a Teflon homogenizer using 1.15% KCl. The homogenate was centrifuged at 12,000×*g* for 25 min at 4 °C. The supernatant was further centrifuged at 100,000×*g* for 90 min at 4 °C to obtain the microsomal fraction. The pellet obtained was suspended in 1.15% KCl. Western blot analysis for CYP3A2 and CYP2C11 was performed according to methods described previously (Kitaichi et al., 2004).

For Western blotting of iNOS, the livers were homogenized with cold 20 mM Tris–HCl buffer (pH 7.2) containing 2 mM phenylmethylsulfonyl fluoride (PMSF, Sigma), 1 mM dithiothreitol (DTT, Sigma) and a protease inhibitor cocktail tablet complete™-mini (Roche Diagnostics, Indianapolis, IN, USA). The homogenate was centrifuged at 11,600×*g* for 30 min at 4 °C. To the supernatant was added Nonidet P40 (Daiichi Kagaku Yakuhin, Tokyo, Japan) to a final concentration of 1%. The protein concentration in the supernatant was measured by Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA, USA), using bovine serum albumin (Sigma) as a standard. Protein (40 μg) was separated by electrophoresis on 6% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and transferred to polyvinylidene (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 4% nonfat dry milk for 30 min at room temperature. The membrane was incubated for 20 h at 4 °C in a solution containing a 1:5000 dilution of monoclonal antibody to iNOS (Transduction Laboratories, Lexington, KY, USA), and washed and incubated in a solution containing a 1:10,000 dilution of horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (Dako, Glostrup, Denmark) for 30 min at room temperature. After washing, the immunoreaction was detected by the enhanced chemiluminescence detection system (ECL, Amersham Biosciences, Piscataway, NJ, USA). To quantify the relative levels of CYP3A2, CYP2C11 and iNOS in each gel, the intensity of the stained bands was measured with the NIH image program (Bethesda, MD, USA).

2.9. Data analysis

Concentration–time data for antipyrine in each rat were analyzed individually using a noncompartmental model. 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_{SYS}) was calculated by dividing the dose by the AUC. The steady state volume of distribution (V_{SS}) was calculated as $V_{SS}=CL_{SYS}\times MRT$, where MRT represents the mean residence time and was calculated as MRT=AUMC/AUC.

2.10. Statistical analysis

All data are expressed as means±S.E.M. Analysis of variance (ANOVA) was used to determine the statistical significance of differences between experimental groups. When F ratios were significant, Scheffé's post-hoc tests between two groups were performed. The 0.05 level of probability was used as the criterion of significance.

3. Results

3.1. Biochemical and histopathological examinations

Data from the biochemical tests are summarized in Table 1. The biochemical parameters showed that the dosage schedule of pioglitazone used in this study did not cause severe liver cell injury. Light micrographs of liver treated with hematoxylin and eosin stain are shown in Fig. 1. Histopathological examination revealed that the hepatocytes of rats treated with endotoxin plus pioglitazone and pioglitazone alone had no evidence of massive necrotic and apoptotic areas. These results indicate that the dosage schedule of pioglitazone in this study does not induce damage to the liver.

3.2. Effect of pioglitazone on endotoxin-induced decrease in antipyrine clearance

We previously reported that the systemic clearance of antipyrine correlated well with hepatic drug-metabolizing enzyme activity in rats treated with endotoxin (Nadai et al., 1998a,b). We thus examined the effect of the pretreatment with pioglitazone on the systemic clearance of antipyrine in endotoxin-treated and untreated rats. The protective effect of pioglitazone against the endotoxin-induced decrease in the systemic clearance of antipyrine is illustrated in Fig. 2. The

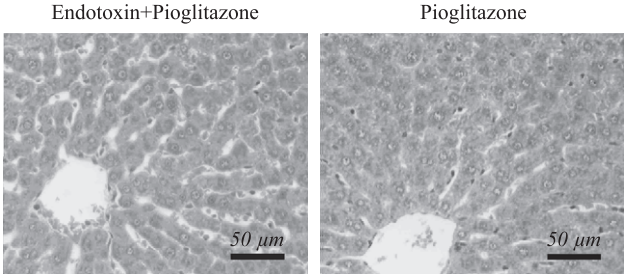

Fig. 1. Light micrograph of rat liver treated with endotoxin plus pioglitazone (left) and pioglitazone alone (right). Pioglitazone (10 mg/kg, 4 times at 10-min intervals) was administered 1 h before injection of endotoxin (1 mg/kg).

systemic clearance of antipyrine (0.24±0.02 l/h/kg) was significantly decreased in rats 24 h after injection of endotoxin, compared to that in control rats (0.48±0.03 l/h/kg). Pretreatment with pioglitazone (1–3 times at 10-min intervals) had no effect on the endotoxin-induced decrease in the systemic clearance of antipyrine. However, pretreatment with pioglitazone (4 times at 10-min intervals) significantly protected the endotoxin-induced decrease in the systemic clearance of antipyrine (0.37±0.03 l/h/kg). No significant differences were observed in the systemic clearance of antipyrine between the control and rats pretreated with pioglitazone alone (4 times at 10-min intervals). On the other hand, the steady state volume of distribution of antipyrine in control, endotoxin alone, endotoxin plus pioglitazone (4 times at 10-min intervals) and pioglitazone alone (4 times at 10-min intervals) were 1.06±0.06, 0.93±0.09, 0.95±0.02 and 0.99±0.03 l/kg, respectively. There were no significant differences among these values, indicating that the decreased systemic clearance of antipyrine by endotoxin was not caused by alterations in the distribution volume.

3.3. Effect of pioglitazone on endotoxin-induced decrease in CYP3A2 and CYP2C11

We examined the effect of pioglitazone on endotoxin-induced decreases in the expression of CYP3A2 and CYP2C11 proteins in the liver. As shown in Fig. 3, the protein levels of CYP3A2 and CYP2C11 in rats 24 h after injection of endotoxin were significantly down-regulated to approximately 50 and 70% of those in control rats, respectively. Pretreatment with pioglitazone completely

Table 1
Biochemical test data for endotoxin alone-, endotoxin plus pioglitazone-, pioglitazone alone-treated and untreated rats

Treatment	Total protein (g/dl)	Albumin (g/dl)	AST (IU/l)	ALT (IU/l)	ChE (IU/l)
Control	5.5±0.4	2.2±0.1	92±7.0	24±2.1	82±3.4
Endotoxin	5.0±0.3	2.1±0.1	109±13.2	45±16	83±4.7
Endotoxin+pioglitazone	5.1±0.2	2.0±0.1	72±11	20±11	80±1.2
Pioglitazone	4.7±0.2	1.8±0.1	71±8.8	19±2.3	82±3.2

Pioglitazone (10 mg/kg, 4 times at 10-min intervals) was administered 1 h before endotoxin injection (1 mg/kg) or saline. Blood samples were collected 24 h after injection of endotoxin. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ChE, cholinesterase. Each value is the mean±S.E.M. (n=3).

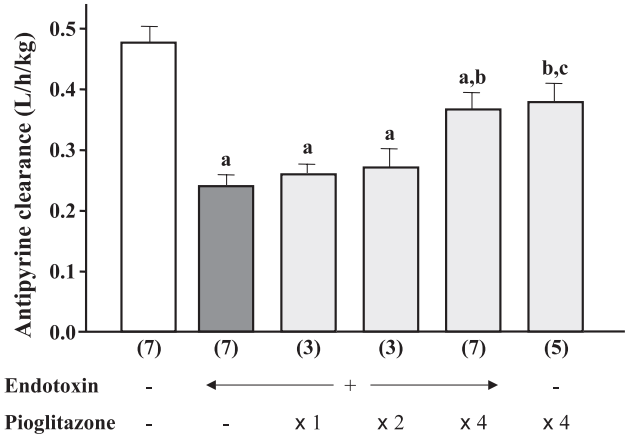

Fig. 2. Effect of pioglitazone on systemic clearance of antipyrine in rats treated with or without endotoxin. Endotoxin (1 mg/kg) was injected 1 h after final injection of pioglitazone (10 mg/kg). Twenty-four hours after endotoxin injection, antipyrine (20 mg/kg) was injected intravenously and blood samples were collected at designated intervals. The number of animals per group is presented in parentheses. [a,b]Significantly different from control and endotoxin-treated rats, respectively ($P<0.01$). [c]Not significantly different from the control.

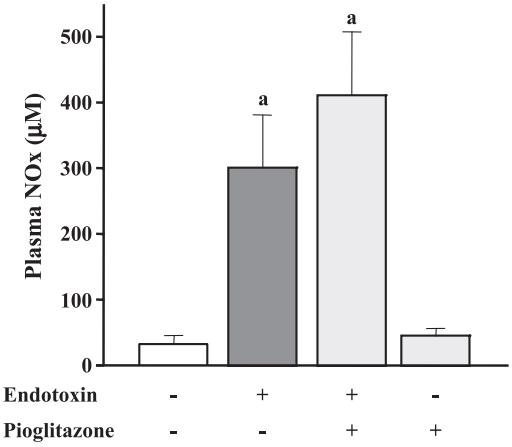

Fig. 4. Effect of pioglitazone on production of NO in rats treated with or without endotoxin. Pioglitazone (10 mg/kg) was administered 1 h before endotoxin injection (1 mg/kg). Twelve hours after endotoxin injection, blood samples were collected to measure concentrations of NOx in plasma. Each column represents the mean±S.E.M. (n=4–6). [a]Significantly different from the control ($P<0.01$).

protected the endotoxin-induced down-regulation of CYP3A2 and tended to inhibit the down-regulation of CYP2C11. The treatment with pioglitazone alone had no effect on the protein expression of either CYP3A2 or CYP2C11.

3.4. Effect of pioglitazone on endotoxin-induced overproduction of NO

We examined the effect of pioglitazone on endotoxin-induced overproduction of NO in plasma. We previously reported that concentrations of NOx in plasma reached maximum levels around 12 h after injection of endotoxin (Kitaichi et al., 1999). We thus measured the concentrations of NOx in plasma 12 h after endotoxin injection. As shown in Fig. 4, the plasma concentrations of NOx in rats treated with endotoxin alone (approximately 300 μM) were approximately 10-fold the basal levels. Pretreatment with pioglitazone had no effect on endotoxin-induced overproduction of NOx in plasma. In addition, treatment with pioglitazone alone (4 times at 10-min intervals) did not show any effect on the NOx levels in plasma.

3.5. Effect of pioglitazone on endotoxin-induced up-regulation of iNOS

As shown in Fig. 5A, the highest protein levels of iNOS were observed 6 h after injection of endotoxin and thereafter the protein levels decreased time dependently. Thus, the effect of pioglitazone on the levels of iNOS expression in the liver was assessed 6 h after injection of endotoxin. Pretreatment with pioglitazone significantly

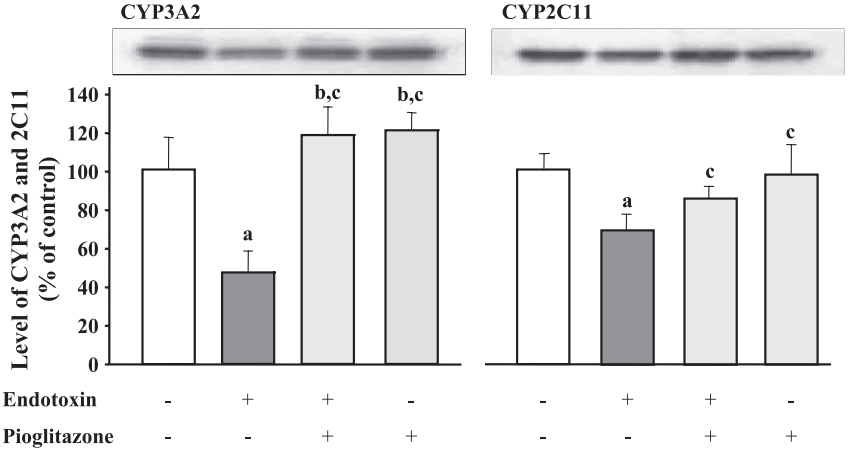

Fig. 3. Effect of pioglitazone on expression of CYP3A2 and 2C11 proteins in liver of rats treated with or without endotoxin. Twenty-four hours after endotoxin injection (1 mg/kg) with or without pioglitazone, the liver was collected to evaluate CYP3A2 expression using Western blot. Each column represents the mean±S.E.M. (n=4). [a,b]Significantly different from control and endotoxin-treated rats, respectively ($P<0.05$). [c]Not significantly different from the control.

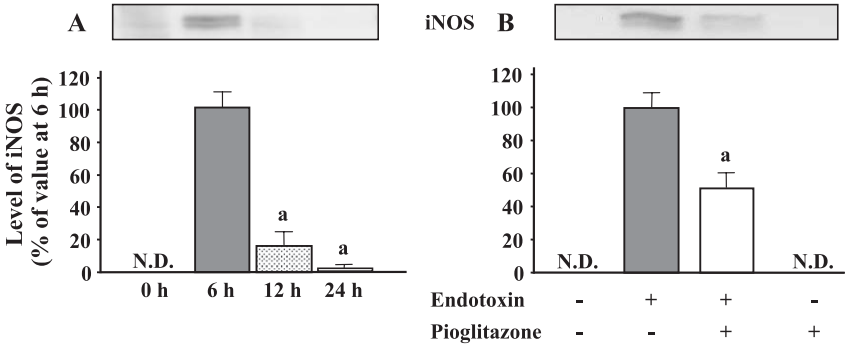

Fig. 5. Time course effect of endotoxin on hepatic iNOS expression and the effect of pioglitazone on iNOS expression in rats treated with or without endotoxin. (A) Zero, six, twelve and twenty-four hours after endotoxin injection (1 mg/kg). (B) Six hours after endotoxin injection (1 mg/kg) with pioglitazone (40 mg/kg). The level of iNOS protein in the liver was measured by Western blot. Each column represents the mean±S.E.M. (n=3–4). [a]Significantly different from rats 6 h after endotoxin injection (P<0.01).

protected the endotoxin-induced protein levels of iNOS by approximately 50% (Fig. 5B). Treatment with pioglitazone alone had no effect on the protein levels of iNOS in the liver.

4. Discussion

CYP-dependent drug-metabolizing enzymes, which mainly exist in the liver and small intestine (Kolars et al., 1994), play a major role in phase I metabolism and inactivation of endogenous and exogenous compounds in humans and animals. In particular, the reduction of hepatic CYP-dependent drug-metabolizing enzyme activity caused by triggers such as drugs, disease states, infection and inflammation causes severe side-effects by increasing drug concentrations in plasma. For example, infection causes a reduction of CYP-dependent drug-metabolizing enzyme activity in humans and animals (Morgan, 1997) and is accompanied by overproduction of various mediators (Roe et al., 1998; Sewer et al., 1996; Warren et al., 1999). Considering that pioglitazone is used to control hyperglycemia in particularly insulin-resistant patients with type II diabetes and is prescribed in combination with other drugs, and that diabetic patients are easily susceptible to bacterial infections, it is crucial to assess the effect of pioglitazone on CYP-dependent drug-metabolizing enzymes in vivo and to investigate whether pioglitazone causes CYP-mediated drug interactions with the combined drugs that are mainly metabolized. However, it is still unclear whether pioglitazone modifies CYP-mediated drug-metabolizing enzyme activity. In the present study, we focused on the effect of pioglitazone on pathophysiological changes in endotoxemia and investigated whether pioglitazone could prevent the reduction of hepatic drug-metabolizing enzyme activity and down-regulation of the expression of CYP3A2 and CYP2C11 induced by endotoxin.

There are numerous reports regarding the mechanisms responsible for endotoxin-induced down-regulation of hepatic CYP-mediated drug-metabolizing enzyme activity. The overproduction of NO by endotoxin reportedly directly inactivates hepatic CYP activity (Minamiyama et al., 1998; Takemura et al., 1999). These results suggest that NO plays an important role in the decreased hepatic CYP activity in endotoxemia, especially in the early phase. Our group also reported that NO is partially responsible for endotoxin-induced reduction of hepatic drug-metabolizing enzyme activity (Kitaichi et al., 1999). Conversely, the down-regulation of CYP mRNA and protein by endotoxin was found to be NO-independent (Sewer et al., 1998; Sewer and Morgan, 1998). Morgan (2001) noted that the discrepancy between NO-dependent and -independent down-regulation by endotoxin is due to differences in the dose of endotoxin used. Considering that endotoxin derived from different bacterial sources has differential cytokine-inducing activity (Flad et al., 1993; Frieling et al., 1997; Mathiak et al., 2003; Netea et al., 2001) and that the action of the *K. pneumoniae* endotoxin used in this study on NO production was very much stronger than that of the *E. coli* endotoxin used in their studies although the same dose was used (unpublished data), the discrepancy may be due to a bacterial strain difference between *K. pneumoniae* and *E. coli*. In addition, our studies evaluated not only the level of CYP proteins, but also the function of CYP-dependent metabolism using the probe, antipyrine, whereas the other studies did not evaluate this function. Further studies are needed to elucidate the precise role of NO in CYP down-regulation by endotoxin.

More recently, Beigneux et al. (2000) published an interesting study indicating that PPAR-γ mRNA expression and PPAR-γ activity in the liver of hamsters is reduced by endotoxin, suggesting the possibility that activation of PPAR-γ suppresses endotoxin-induced inflammatory responses in the liver. Our interest in the effect of pioglitazone on endotoxin-induced reductions in the hepatic CYP-dependent drug-metabolizing enzyme activity and expression of CYP3A2 and CYP2C11 proteins was stimulated by reports that PPARγ ligands can modulate inflammatory response (Jiang et al., 1998; Reilly et al.,

2001; Ricote et al., 1998; Uchimura et al., 2001). First, we examined whether pioglitazone could protect the reduction of CYP activity induced by endotoxin. Antipyrine clearance experiments showed that pretreatment with pioglitazone significantly reduced the decrease in systemic clearance of antipyrine in rats 24 h after endotoxin injection, with no histopathological changes in the liver. This would suggest that pioglitazone might have a protective effect against an endotoxin-induced decrease in the hepatic CYP-dependent metabolizing enzyme activity. To our knowledge, this is the first in vivo report that PPAR-γ ligand reverses the decrease in hepatic CYP activity by endotoxin.

Interestingly, the present study found that pretreatment with pioglitazone did not begin to restore to basal levels the elevated NO levels induced by endotoxin, although the overexpression of iNOS protein by endotoxin was significantly restored by pretreatment with pioglitazone. The restorative effect of pioglitazone against the iNOS levels elevated by endotoxin is consistent with the results of Jiang et al. (1998) and Ricote et al. (1998). Endotoxin reportedly reduces the expression of endothelial NO synthase (eNOS) in peritoneal tissues (Arriero et al., 2000) and PPAR-γ agonists amplify cytokine-stimulated iNOS expression in mesangial cells and vascular smooth muscle cells (Carcillo et al., 2003; Reilly et al., 2001). Based on these observations, we propose that PPAR-γ ligands have cell or tissue specificity for iNOS expression. The lack of effect of pioglitazone on the NO levels elevated by endotoxin observed in the present study may be explained by the overproduction of NO mediated by iNOS in other tissues or cells rather than liver. However, it remains unclear whether the overproduction of NO in the liver plays a pivotal role in endotoxin-induced suppression of the hepatic drug-metabolizing enzyme activity.

Antipyrine is the preferred probe for determining hepatic oxidative metabolic capacity in humans and animals (Balani et al., 2002; Carcillo et al., 2003; Kitaichi et al., 1999; Nadai et al., 1998a,b), since it is almost entirely metabolized in the liver, and its hepatic clearance is independent of both hepatic blood flow and protein binding potency. Antipyrine metabolism in humans is associated with at least six hepatic CYP isoenzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18 and CYP3A4), three of which (CYP3A4, CYP2C8 and CYP2C9) are closely related to antipyrine metabolism rather than to other isoenzymes (Engel and Cosme, 1996). Second, we examined the effect of pioglitazone on endotoxin-induced down-regulation of CYP3A2 and CYP2C11 protein in the liver. Pretreatment with pioglitazone at a dose that prevents endotoxin-induced down-regulation of CYP did not change the protein levels of CYP3A2 and CYP2C11 in normal rats, although pioglitazone is predominantly metabolized via CYP3A4 and CYP2C8 (information from Takeda Pharmaceuticals America, 2000). Moreover, unlike troglitazone, pioglitazone has no effect on CYP3A4 activity either in vitro or in vivo (Nowak et al., 2002; Yamazaki et al., 2000). Indeed, there is no report that PPAR response elements are present at the 5′-upstream region of the gene encoding CYP3A and CYP2C (Savas et al., 1999). On the basis of these observations, it is unlikely that the protective effect of pioglitazone against endotoxin-induced reduction of CYP activity is caused by the direct induction of CYP, although the mechanism by which pioglitazone ameliorates the down-regulation of hepatic CYP3A2 and 2C11 by endotoxin remains unclear at present.

Braissant et al. (1996) suggest that PPAR-γ is not expressed in rat hepatocytes. We therefore presume that the amelioration of endotoxin-induced down-regulation of hepatic CYP3A2 and CYP2C11 protein by pretreatment with pioglitazone might be due to indirect action by pioglitazone. According to previous reports (Cassatella et al., 1993; Crawford et al., 1997; Evans et al., 1993; Jiang et al., 1998; Ricote et al., 1998), it is postulated that this phenomenon might be associated with modulation of endotoxin-induced pro-inflammatory mediator levels (such as TNF-α, interleukin-1β and interleukin-6) by pioglitazone.

Pioglitazone is shown to be a weak PPAR-α activator in addition to a selective PPAR-γ activator (Sakamoto et al., 2000). It is suggested that PPAR-α plays an important role in hepatic CYP down-regulation by endotoxin (Barclay et al., 1999). A preliminary experiment was performed to confirm whether the PPAR-α ligand, fenofibrate, affects the endotoxin-induced systemic clearance of antipyrine. We found that fenofibrate at the dosage schedule which is able to activate PPR-α in hepatocytes (320 mg/kg/day for 2 days) (Kockx et al., 1999), had no effect on the endotoxin-induced decrease in the hepatic drug-metabolizing enzyme activity (data not shown). These results may suggest that PPAR-γ activation, but not PPAR-α activation, by pioglitazone might modulate endotoxin-induced down-regulation of hepatic CYP3A2 and CYP2C11 protein levels.

In conclusion, the present study is the first to report that pioglitazone reduces the endotoxin-induced impairment of hepatic drug-metabolizing enzyme activity and down-regulation of hepatic CYP3A2 and 2C11 protein by PPAR-γ activation. Further study is needed to elucidate the precise mechanism by which pioglitazone reduces the down-regulation of hepatic CYP3A2 and 2C11 expression induced by endotoxin.

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