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EFFECTS OF INTERLEUKIN 1α ON THE ACTIVITIES AND GENE EXPRESSIONS OF THE CYTOCHROME P450IID SUBFAMILY

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Abstract—The mechanism by which recombinant human interleukin 1α (rhIL-1α) inhibits the activities of drug-metabolizing enzymes of rat liver microsomes, especially debrisoquine monooxygenase and bufuralol monooxygenase (both cytochrome P450IID supported reactions), as well as other enzymes, was investigated by injecting IL-1α into rats. rhIL-1α suppressed the activities of various P450-linked monooxygenase systems such as aminopyrine *N*-demethylase, benzphetamine *N*-demethylase, and 7-ethoxycoumarin *O*-deethylase. It also suppressed the activities of debrisoquine monooxygenase and bufuralol monooxygenase. On the other hand, IL-1α had little effect on the activity of *p*-nitroanisole *N*-demethylase. The suppression of debrisoquine monooxygenase and bufuralol monooxygenase activities was caused by a decrease in the amounts of immunoreactive P450IID protein and its mRNA. The reduction rates in the level of immunoreactive P450IID protein and its mRNA were comparable. These results suggest that at the mRNA level, the enzymatic activities of debrisoquine monooxygenase and bufuralol monooxygenase are down-regulated by IL-1α.

Interleukin-1 (IL-1)§ is produced by monocytes, macrophages and some other cells in inflammatory reactions and plays many important roles as one of the inflammatory cytokines. Interleukin-1 induces acute phase proteins such as amyloid A and haptoglobulin by working on the hepatocytes [1]. Recently, it was reported that IL-1 suppresses the activity and content of the P450-linked monooxygenase system of mouse liver microsomes in *in vivo* [2, 3] and *in vitro* [4] experiments. Furthermore, Wright and Morgan [5] reported that IL-1α and glucocorticoids are important mediators in the suppression of the gene expression of P450IIC12 in the liver. However, the suppressive effects of IL-1 on the enzymatic activities and the gene expressions of P450IID (P450db), a subfamily member and an important drug-metabolizing enzyme, have not been clearly elucidated. Cytochrome P450IID is a constitutive P450 isozyme that is related to drug metabolism and is characterized as a new gene subfamily of debrisoquine 4-monooxygenase. Recently, a lot of clinical attention has been given to the antibody to P450db1. This antibody can be detected in the serum of patients with autoimmune hepatitis type II as the autoantibody [6, 7].

The effects of IL-1α on the activity and the content of P450db1 were investigated by performing quantitative analyses of enzymatic activities, immunoreactive P450IID proteins and its mRNA of liver microsomes prepared from IL-1-treated and untreated rats. Furthermore, we tested whether the suppressive effects of IL-1 on the activity and content of the P450-linked monooxygenase system are common phenomena with respect to all P450 isozymes.

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§ Abbreviations: IL-1, interleukin 1; rhIL-1, recombinant human interleukin 1; P450, cytochrome P450; and P450db1, cytochrome P450 debrisoquine.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing about 100 g (5 weeks old) were used throughout this study. They were maintained in a controlled lighting cycle (12 hr on, 12 hr off) and were deprived of food and water overnight before surgery.

Chemicals. rhIL-1α was a gift from the Dainippon Pharmaceutical Co., Ltd., Tokyo, Japan. NADPH, proteinase K, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, aminopyrine, benzphetamine and 7-ethoxycoumarin were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Debrisoquine, 4-hydroxydebrisoquine, bufuralol, and 1′-hydroxybufuralol were gifts from Dr. H. Fukui of the Department of Pharmacology at the Osaka University Medical School. The Histofine SAB-PO kit (biotinylated goat anti-rabbit immunoglobulin, peroxidase-labeled streptavidin) used for Western blot analysis was purchased from the Nichirei Chemical Co. (Tokyo, Japan). All other chemicals of the highest commercially available grade were purchased from Wako Pure Chemicals (Osaka, Japan) and the Nacalai Chemical Co. (Kyoto, Japan).

Treatment of IL-1. Solutions of rhIL-1α (1.3 and 2.6 μg) were prepared and dissolved in 1 mL saline immediately prior to use. rhIL-1α was administered

i.p. to the rats and 1 mL saline without rhIL-1α was administered to the control rats.

Preparation of liver microsomes. Twenty-four hours after the administration of rhIL-1α, the rats were decapitated and their livers were washed carefully via the portal vein with 50 mM ice-cold potassium phosphate buffer (pH 7.4) containing 1.15% (w/v) potassium chloride to remove as much blood as possible. The microsomes were immediately prepared at below 4° by the method of Mitoma *et al.* [8]. The microsomes were then suspended in 50 mM potassium phosphate buffer (pH 7.4) and used for the assays of enzymatic activity.

Protein contents. Protein concentration was determined by the method of Lowry *et al.* [9] using bovine serum albumin as a standard. A value of $6.68 \times 10^3\ M^{-1}\ cm^{-1}$ at 280 nm was used as the molar extinction coefficient of bovine serum albumin [10].

P450 content. P450 content was determined spectrophotometrically by a CO-reduced difference spectrum with a molar extinction coefficient of $91 \times 10^3\ M^{-1}\ cm^{-1}$ at 450 – 490 nm [11].

Enzymatic activity. The activities of aminopyrine *N*-demethylase, benzphetamine *N*-demethylase and *p*-nitroanisole *N*-demethylase were determined in liver microsomes by measuring the formation of formaldehyde according to the method of Nash [12]. The activity of 7-ethoxycoumarin *O*-deethylase of liver microsomes was determined by the method of Greenlee and Poland [13]. The enzymatic reactions described above were conducted at 37° for 5 min. The activities of debrisoquine monooxygenase and bufuralol monooxygenase were determined by the methods of Kronbach *et al.* [14] and Matsuo *et al.* [15]. The enzymatic reaction was conducted at 37° for 40 min. One milligram of microsomal protein in a 1 mL reaction system was used to measure the activities of drug-metabolizing enzymes. All measurements of the enzymatic activities were done in 50 mM potassium phosphate buffer (pH 7.4) containing 0.2 mM NADPH and an NADPH-regenerating system (5 mM glucose-6-phosphate, 0.5 μM glucose-6-phosphate dehydrogenase and 4 mM $MgCl_2$). These enzymatic activities were confirmed to be dependent on the reaction time and the concentration of microsomal protein.

Western blot analysis. Serum of an autoimmune hepatitis patient containing LKM-1 autoantibody (P450db1 antibody) was used for the antibody to immunoreactive P450IID protein. This serum was a gift from Dr. Michael P. Manns of the Department of Internal Medicine (1st Division) at the University of Mainz, Germany. The antibody to P450IID protein (P450db1) in this serum has already been confirmed by examining the expressed fusion protein of P450db1 cDNA in *Escherichia coli* [6]. It has been reported that the P450db1 antibody in the serum of an autoimmune hepatitis patient can recognize P450db1 protein of rat liver microsomes [16, 17]. Therefore, P450db1 antibody in the serum used in this experiment would also be able to react with P450db1 specifically. Five micrograms of microsomes was used for the Western blot analysis. Bio-Rad low range marker proteins were used for the standard markers of molecular weights of proteins.

RNA slot blot hybridization analyses. Liver specimens of about 500 mg in a mixed solution containing 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol and 0.5% sarcosyl were incubated with 0.2 mg proteinase K at 37° for 12 hr. After washing out the specimen using a disposable syringe with 21 gauge needle, RNA extractions from livers were conducted using the method of Chomczynski and Sacchi [18]. RNA (5 μg) was used for the slot blot hybridization. Bovine P450IID cDNA, cloned and sequenced* and found to be approximately 70% homologous to the rat P450IID subfamily, was used as the probe. Under the hybridization conditions that were used, at least 70% homologous to the cDNA probe, which also means the same subfamily, i.e. a P450IID subfamily member, should be picked out. Therefore, the rat P450IID subfamily member was detected under the hybridization conditions used. The labeling of P450IID cDNA with [^{32}P]dATP was carried out by the method of nick translation [19]. Hybridization was conducted in 4× SETDS buffer [0.6 M potassium chloride, 120 mM Tris–HCl (pH 7.5), 8 mM EDTA, 10× Denhardt, 0.1% (w/v) sodium dodecyl sulfate (SDS)] containing 50 μg/mL heat-denatured salmon sperm DNA at 60° for 24 hr. A single washing was conducted in 4× SETS buffer [0.15 M potassium chloride, 30 mM Tris–HCl (pH 7.5), 2 mM EDTA,

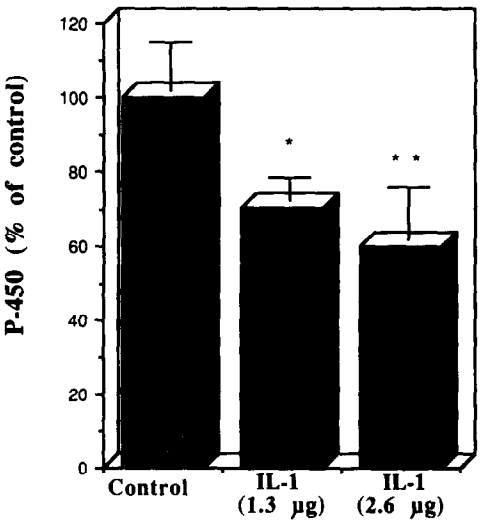

Fig. 1. Effects of rhIL-1α on P450 contents of rat liver microsomes. rhIL-1α (1.3 and 2.6 μg) dissolved in 1 mL saline, and 1 mL saline without rhIL-1α for the control, were administered i.p. to the rats. The P450 contents of liver microsomes after administration of rhIL-1α (1.3 and 2.6 μg/rat) are expressed as a percentage of the values of untreated rat liver microsomes. Each value is the mean ± SD of 4 rats. Values marked with an asterisk(s) were significantly different from the control: *$P < 0.05$ and **$P < 0.01$. The mean P450 content of liver microsomes of untreated rats was 0.82 ± 0.11 nmol/mg microsomal protein.

* Tsuneoka Y, Matsuo Y, Higuchi R and Ichikawa Y, unpublished observations.

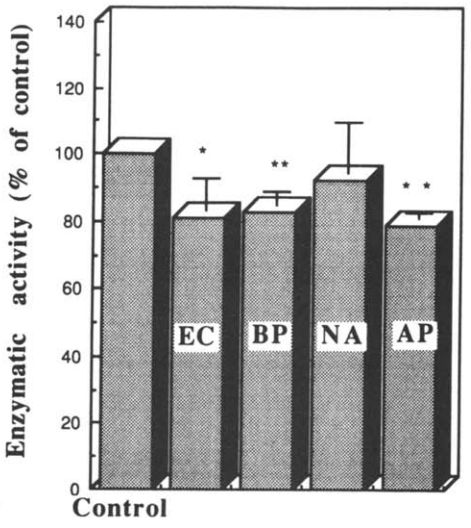

Fig. 2. Effects of rhIL-1α on the activities of benzphetamine *N*-demethylase (BP), aminopyrine *N*-demethylase (AP), *p*-nitroanisole *N*-demethylase (NA) and 7-ethoxycoumarin *O*-deethylase (EC) of rat liver microsomes. rhIL-1α (2.6 μg) dissolved in 1 mL saline, and 1 mL saline without rhIL-1α for the control, were administered i.p. to the rats. Benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *N*-demethylase and 7-ethoxycoumarin *O*-deethylase activities are expressed as a percentage of the values of untreated rat liver microsomes. The means and standard deviations of the specific activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *N*-demethylase and 7-ethoxycoumarin *O*-deethylase of liver microsomes from untreated rats were 4.25 ± 1.30, 5.55 ± 0.34, 2.29 ± 0.32 and 2.79 ± 0.88 nmol/min/mg microsomal protein (pH 7.4 and 37°), respectively. Each value is the mean ± SD of 4 rats. Values marked with an asterisk(s) were significantly different from control: $^*P < 0.05$ and $^{**}P < 0.01$.

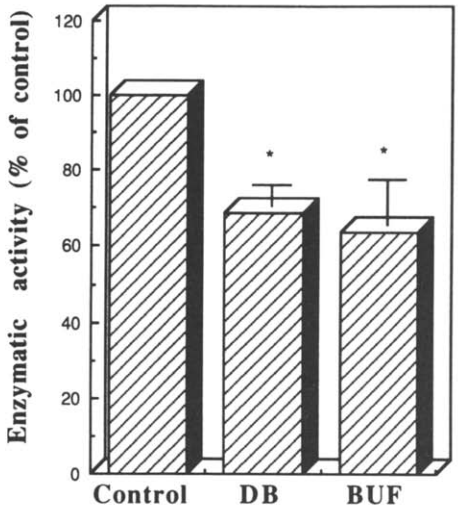

Fig. 3. Effects of rhIL-1α on debrisoquine monooxygenase and bufuralol monooxygenase of rat liver microsomes. rhIL-1α (2.6 μg) dissolved in 1 mL saline, and 1 mL saline without rhIL-1α for the control, were administered i.p. to the rats. The mean specific activities of debrisoquine monooxygenase (DB) and bufuralol monooxygenase (BUF) of liver microsomes from untreated rats were 0.28 ± 0.12 and 0.62 ± 0.23 nmol/min/mg microsomal protein, (pH 7.4 and 37°), respectively. Each value is the mean ± SD of 4 rats. Values marked with an asterisk were significantly different from the control: $^*P < 0.01$.

0.1% (w/v) SDS] followed by a double washing in 1× SETS buffer at 60° for 15 min.

Densitometrical analyses of Western blot and RNA slot blot hybridization. Densitometrical analyses of immunoblots and slot blots were performed using a Shimadzu Dual Wavelength TLC Scanner, model CS 910. Densitometrical analysis of Western blot was done at 550 nm and that of RNA slot blot at 610 nm.

Statistical analysis. Statistical analysis was performed on Macintosh classic Stat-Work software. The statistical significance values in Figs.1–5 were determined using Student's *t*-test.

RESULTS

Figure 1 shows the effects of IL-1α (1.3 and 2.6 μg/rat) on the P450 content of rat liver microsomes. A single administration of 1.3 and 2.6 μg of IL-1α dissolved in 1 mL saline to the rats significantly decreased P450 content to 70 and 59% of the original P450 content of untreated liver microsomes, respectively. The suppression of the P450 content by IL-1α was dose dependent between 0 and 2.6 μg. The mean P450 content of liver microsomes of untreated rats was 0.82 ± 0.11 nmol/mg microsomal protein.

Figure 2 illustrates the effects of IL-1α (2.6 μg/each rat) on several enzymatic activities: benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *N*-demethylase and 7-ethoxycoumarin *O*-deethylase. Administration of 2.6 μg of IL-1α suppressed the activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase and 7-ethoxycoumarin *O*-deethylase to 82, 78 and 81% of the activities of untreated rats, respectively. The activities described above were not affected by the direct addition of IL-1α to the solution of liver microsomes. In contrast, *p*-nitroanisole *N*-demethylase activity was not affected by the administration of IL-1α to rats. The means and standard deviations of the specific activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *N*-demethylase and 7-ethoxycoumarin *O*-deethylase of liver microsomes of untreated rats were 4.25 ± 1.30, 5.55 ± 0.34, 2.29 ± 0.32 and 2.79 ± 0.88 nmol/min/mg microsomal protein, respectively.

Figure 3 depicts the effects of IL-1α (2.6 μg/rat) on the activities of debrisoquine monooxygenase and bufuralol monooxygenase. The administration of IL-1α decreased both activities to approximately 70% of those of untreated rats. To clarify which regulatory step is affected by IL-1α treatment, a quantitative analysis of the amounts of P450IID protein and its mRNA was performed. The means

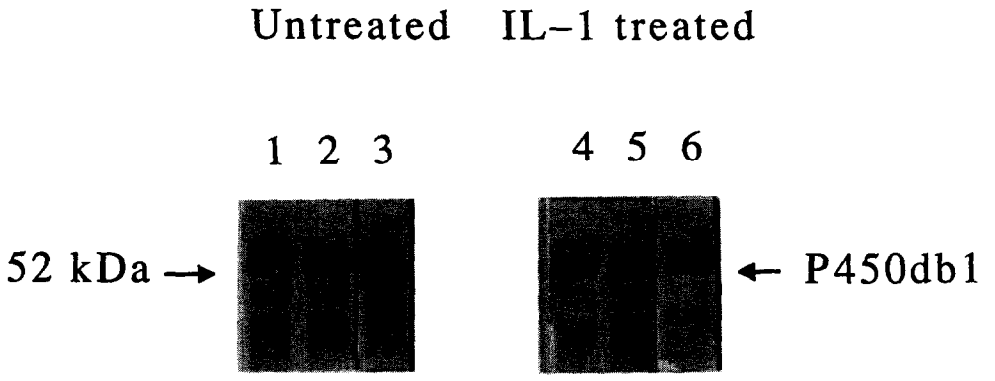

Fig. 4. Western blot analysis of P450dbl from rat liver microsomes. Six samples of individual liver microsomes are shown. P450dbl of liver microsomes was stained using the serum of an autoimmune hepatitis patient containing P450 dbl autoantibody. Microsomal proteins (5 μg) were applied for Western blot analysis. The minimum molecular weights of the stained protein bands were 52 kDa. Lanes 1–3 represent untreated rats, while lanes 4–6 represent IL-1 treated rats, as described in Materials and Methods.

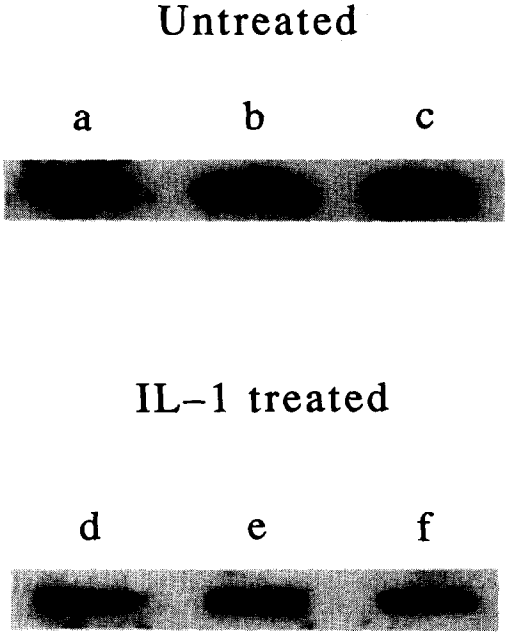

Fig. 5. Slot blot analysis of total RNA extracted from rat liver. Six RNA samples from the individual livers are shown. Bovine P-450IID cDNA was used as a probe. Five micrograms of RNA extracted from livers was applied for slot blot. Lanes a–c represent untreated rats, while lanes d–f represent IL-1 treated rats, as described in Materials and Methods.

and standard deviations of the specific activities of debrisoquine monooxygenase and bufuralol monooxygenase of liver microsomes of untreated rats were 0.28 ± 0.12 and 0.62 ± 0.23 nmol/min/mg microsomal protein, respectively.

Figure 4 shows the Western blot analysis of liver microsomes using the serum containing the P450dbl antibody. The minimum molecular weights of the P450dbl of IL-1-treated and untreated rat liver microsomes were estimated to be identically 52 kDa. By densitometrical analyses of the stained protein bands of Western blot with absorbance at 550 nm, the areas (mm^2) of the absorption peaks of lanes 1–6 in Fig. 4 were 68.0, 81.0, 78.0, 37.5, 48.0, and 43.0, respectively. Significant changes ($P < 0.05$) were observed between the absorption peak areas of untreated rats (lanes 1–3) and those of treated rats (lanes 4–6).

Figure 5 show the results of RNA slot blot hybridization analyses of P450IID mRNA. By the densitometrical analysis of the hybridized bands of RNA slot blot, the areas (mm^2) of the absorption peaks of lanes a–f in Fig. 5 were 80.0, 85.5, 120.0, 44.0, 72.0, and 46.5, respectively. Significant changes ($P < 0.05$) were observed between the peak areas of untreated rats and those of treated rats by the statistical analysis method described in this paper. These results show that the activities of debrisoquine monooxygenase and bufuralol monooxygenase are regulated by IL-1 at the mRNA level.

DISCUSSION

In this study we investigated the effects of IL-1α on P450 contents and activities along with several drug-metabolizing enzymes and gene expressions of drug-metabolizing monooxygenase systems. Shedlofsky *et al.* [2] reported that IL-1α depresses P450 contents and the activities of benzphetamine *N*-demethylase and ethoxyresorufin *O*-deethylase in mouse liver microsomes. Our results with rat liver microsomes were consistent with their findings. Furthermore, the suppressive effects of IL-1α on the activities of aminopyrine *N*-demethylase, ethoxycoumarin *O*-deethylase, debrisoquine monooxygenase and bufuralol monooxygenase were shown

in this study. The suppressive effect of IL-1α on *p*-nitroanisole *N*-demethylase activity was not observed in this study. The significance of this observation awaits further elucidation.

According to a previous report [20], the specific activity of *p*-nitroanisole *N*-demethylase is low when compared with that of other drug-metabolizing enzymes measured in this experiment. Therefore, the effects of IL-1α on the activity of *p*-nitroanisole *N*-demethylase would be slight. It may be difficult to detect the slight change of the activity of *p*-nitroanisole *N*-demethylase. Although it is well known that there is overlapping for the substrate specificity in the enzymatic activities of P450 isozymes, the major specific activity of individual P450 isozymes corresponds to a specific P450 isozyme as follows: the enzymatic activity is associated with P450IID6 for debrisoquine 4-monooxygenation and bufuralol 1′-monooxygenation, P450IIB1 and P450IIC11 for benzphetamine N-demethylation, P450IA1 for 7-ethoxycoumarin O-deethylation, P450IIB1 for aminopyrine N-demethylation, and P450IIC11 for *p*-nitroanisole N-demethylation [21–25]. Our results suggest that IL-1α affects not only one specific isozyme of P450 but also many other P450 isozymes such as IID6, IIB1, IIC11 and IA1.

Furthermore, the effects of IL-1α on P450IID6 protein content and its mRNA level were investigated. The results suggest that the decreased activities of debrisoquine monooxygenase and bufuralol monooxygenase are parallel with the amount of immunoreactive P450IID protein, and that the decrease of P450IID protein was caused by the decrease of the P450IID mRNA level.

Both IL-1 and IL-6 are major cytokines that are related to inflammatory reactions. Recently, it was reported that the contents of P450IIB1/2 and P450IIC12 mRNA were decreased by IL-1 and IL-6. Furthermore, IL-1 produces a more rapid suppression of the expression of P450IIC12 than IL-6 [5, 26]. From these reports, as well as the results obtained from our study, it is probable that the gene expressions of many kinds of drug-metabolizing monooxygenases are depressed by IL-1 produced in inflammatory reactions. In these experiments, the reduced rates of immunoreactive P450IID protein and P450IID mRNA were comparable, suggesting that IL-1α does not alter significantly the rate of translation from P450IID mRNA to P450IID proteins but affected the mRNA level which was determined by the balance of the rate of mRNA synthesis and the stability of mRNA. In conclusion, the P450IID gene expression was suppressed by IL-1α.

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