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Metabolism of medroxyprogesterone acetate (MPA) via CYP enzymes in vitro and effect of MPA on bleeding time in female rats in dependence on CYP activity in vivo

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Abstract

Medroxyprogesterone acetate (MPA) is a drug commonly used in endocrine therapy for advanced breast cancer, although it is known to cause thrombosis as a serious side effect. Recently, we found that cytochrome P450 3A4 (CYP3A4) mainly catalyzed the metabolism of MPA via CYP in human liver microsomes. However, the metabolic products of MPA in humans and rats have not been elucidated. In addition, it is not clear whether thrombosis could be induced by MPA itself or by its metabolites. In this study, we determined the overall metabolism of MPA as the disappearance of the parent drug from an incubation mixture, and identified the enzymes catalyzing the metabolism of MPA via CYP in rats. Moreover, the effects of CYP-modulators on MPA-induced hypercoagulation in vivo were examined. Intrinsic clearance of MPA in rat liver microsomes was increased by treatment with CYP3A-inducers. The intrinsic clearance of MPA in liver microsomes of rats treated with various CYP-inducers showed a significant correlation with CYP3A activity, but not CYP1A activity, CYP2B activity or CYP2C contents. Among the eight recombinant rat CYPs studied, CYP3A1, CYP3A2 and CYP2A2 catalyzed the metabolism of MPA. However, since CYP3A2 and CYP2A2 are male-specific isoforms, CYP3A1 appears to be mainly involved in the metabolism of MPA in liver microsomes of female rats. In an in vivo study, pretreatment of female rats with SKF525A, an inhibitor of CYPs including CYP3A1, significantly ($p < 0.05$) enhanced MPA-induced hypercoagulation, whereas pretreatment with phenobarbital, an inducer of CYPs including CYP3A1, reduced it. These findings suggest that CYP-catalyzed metabolism of MPA is mainly

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catalyzed by CYP3A1 and that MPA-induced hypercoagulation is predominantly caused by MPA itself in female rats.

Keywords: MPA; P450; Rat liver microsomes; cDNA-expression system; Hypercoagulation

Introduction

Medroxyprogesterone acetate (MPA) is one of the drugs most commonly used in endocrine therapy for advanced or recurrent breast cancer and endometrial cancer. However, it is known that MPA can cause serious adverse effects such as thrombosis as well as other side effects such as weight gain, hypertension, nausea and cushingoid effects (Etienne et al., 1992). Although a higher frequency of toxicity has been seen at higher doses (Etienne et al., 1992), it is not clear whether MPA actually contributes to the occurrence of thrombosis, because thrombosis is caused by many factors, including tumors, surgery and genetic mutations of some coagulation factors (Rosendaal, 1999). Recently, it has been reported that MPA shortened bleeding time in rats in a dose-dependent manner (Nobukata et al., 1999). These results suggest that MPA itself and/or its metabolites might stimulate blood coagulation.

MPA exhibits low oral bioavailability (< 10%), which may be due to numerous factors, including metabolism in the intestinal mucosa and liver (Stockdale and Rostom, 1989). In fact, MPA has been shown to undergo extensive and rapid metabolism in humans (Stockdale and Rostom, 1989) and in experimental animals (Rautio et al., 1985). Recently, we reported that MPA was metabolized by cytochrome P450 3A4 (CYP3A4) in human liver microsomes (Kobayashi et al., 2000). This finding agrees with the observation reported by Ohtsu et al. (1998) that the plasma concentration of MPA was lower than that of MPA alone when dexamethasone (DEX), a CYP3A inducer (Pichard et al., 1990; Morris and Davila, 1996) or phenobarbital (PB), an inducer of CYP2B, CYP2C and CYP3A (Waxman and Azaroff, 1992) was coadministered with MPA in patients with breast cancer. Similarly, PB administered with MPA to rats greater decrease in the plasma concentration of MPA, while SKF525A (a CYP inhibitor) administered with MPA to rats caused a smaller decrease in the plasma concentration of MPA compared to that in the case of administration of MPA alone (Saarni et al., 1983). These results suggest that plasma decay of MPA depends mainly on the metabolism of MPA by CYP, although it is not clear which CYP isoform(s) is responsible for the metabolism of MPA in rats.

Since the metabolic products of MPA in rats have not been elucidated, we determined the metabolism of MPA as the disappearance of the parent drug from an incubation mixture, and identified the rat CYP isoforms involved in the CYP-catalyzed metabolism of MPA by using liver microsomes of DEX-, PB- or β-naphthoflavone (BNF, a CYP1A inducer; Daujat et al., 1992; Morris and Davila, 1996)-treated female rats, and microsomes from baculovirus-infected insect cells expressing individual rat CYP isoforms in the present study. Since CYP1A, CYP2B, CYP2C and CYP3A are major CYP isoforms in female rat liver, their probe activities or contents in liver microsomes were determined. Moreover, we examined the effects of SKF525A and PB on change in bleeding time by a single po administration of MPA in female rats to elucidate whether MPA itself or its metabolites stimulates blood coagulation.

Methods

Chemicals

MPA was obtained from Pharmacia-Upjohn (Tokyo, Japan). Prazepam and aminopyrine were obtained from Nippon Roche (Tokyo, Japan). Furafylline and sulfaphenazole were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Ketoconazole was obtained from Janssen Pharmaceutica (Beerse, Belgium). 6β-Hydroxytestosterone was purchased from Ultrafine Chemicals (Manchester, UK). Formaldehyde standard solution was purchased from Kanto Chemicals (Tokyo, Japan). BNF, 7-benzyloxyresorufin, 7-ethoxyresorufin, methoxyresorufin, resorufin and SKF525A were purchased from Sigma (St. Louis, MO). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and $NADP^+$ were purchased from Oriental Yeast (Tokyo, Japan). DEX, PB, testosterone, HPLC-grade acetonitrile and methanol, and other regents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

All recombinant rat CYPs expressed in insect cells (CYP1A2, CYP2A2, CYP2B1, CYP2C6, CYP2C13, CYP2D1, CYP3A1 and CYP3A2) were obtained from Gentest (Woburn, MA). All recombinant CYPs were coexpressed with rat NADPH-CYP oxydoreductase (OR). Recombinant CYP2A2, CYP2B1, CYP2C6, CYP2C13, CYP3A1 and CYP3A2 were coexpressed with cytochrome b_5. Control microsomes were from insect cells infected with wild-type baculovirus.

Animals

Female Wistar rats were purchased from Japan SLC (Shizuoka, Japan). The rats were maintained under conditions of controlled temperature and light, with access to food and water ad libitum. They were 5 weeks old for preparation of microsomes and 7 weeks old for experiments in vivo.

Rat liver microsomes

Animals were injected intraperitoneally with either PB (one injection/day, 100 mg/kg dissolved in saline, n = 3) or BNF (one injection/day, 25 mg/kg dissolved in corn oil, n = 3) for 4 consecutive days. One rat was treated with DEX (one injection/day, 200 mg/kg suspended in corn oil) for 2 days and was subsequently treated with reduced DEX (one injection of 100 mg/kg). Rats treated with saline (n = 3) or corn oil (n = 3) were used as controls. One day after the final treatment, rats were killed, and the livers were removed. The livers were immediately frozen and stored at − 80 °C.

Rat liver microsomes were prepared by differential centrifugation and were stored at − 80 °C until use. Protein concentrations were measured using a DC protein assay kit (Bio-Rad, Hercules, CA).

MPA metabolism in rat liver microsomes

Since the metabolic products of MPA in humans and rats has not been elucidated, microsomal activity for MPA metabolism was determined by measuring the disappearance rate of MPA from an incubation medium with rat liver microsomes as reported previously (Kobayashi et al., 2000). Briefly, the basic incubation medium contained 0.1 mg protein/mL of rat liver microsomes, 0.1 mM EDTA, 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.5 mM $NADP^+$, 2.0 mM glucose-6-phosphate, 1 IU/ml of glucose-6-phosphate dehydrogenase, and 4 mM $MgCl_2$), and 0.25 μM MPA, in a

final volume of 250 μL. MPA was added to the incubation mixture at a final acetonitrile concentration of 1%. The mixture was incubated at 37 °C for 0, 7, 15 and 30 min. After the reaction was stopped by adding 100 μL of cold acetonitrile, 50 μL of prazepam (0.25 μg/ml in methanol) was added as an internal standard. The mixture was centrifuged at 1700 × *g* for 20 min, and 100 μL of the supernatant was analyzed using a Hitachi HPLC system (Tokyo, Japan) consisting of an L-7100 pump, an L-7400 UV detector, an L-7200 autosampler, a D-7500 integrator, and a CAPCELL PAK C_{18} UG120 column (4.6 × 250 mm, 5 μm; Shiseido, Tokyo, Japan). The mobile phase consisted of 10 mM phosphate/acetonitrile (40/60, v/v) with a flow rate of 1.0 ml/min. The eluate was monitored at a wavelength of 240 nm. The disappearance amount of MPA in the medium incubated at 37 °C with microsomes in the presence of the NADPH-generating system was determined as percentage of the initial amount of MPA in the medium without incubation.

In a preliminary study, MPA less than 0.25 μM disappeared from the incubation mixture at a first-order rate. Therefore, we used 0.25 μM MPA as the substrate concentration to determine the disappearance amount of MPA throughout the study.

Intrinsic clearance of MPA

The intrinsic clearance of MPA in rat liver microsomes was estimated from the volume of incubation medium and elimination rate constant using the following equation: intrinsic clearance = elimination rate constant X volume of incubation medium. The elimination rate constant of MPA in the incubation medium was calculated by regression analysis of semilogarithmic plots.

Inhibition study

The effects of furafylline (an inhibitor of CYP1A; Newton et al., 1995), sulfaphenazole (an inhibitor of CYP2C; Newton et al., 1995) and ketoconazole (an inhibitor of CYP3A; Newton et al., 1995) on the disappearance of MPA at a substrate concentration of 0.25 μM were studied using liver microsomes prepared from untreated rats. The concentrations of inhibitors used were 0.1 and 1 μM for ketoconazole and 1 and 10 μM for furafylline and sulfaphenazole. All inhibitors were added to the incubation medium at a final acetonitrile concentration of 1%.

Assay with recombinant CYPs

Microsomes from baculovirus-infected insect cells expressing CYP1A2 (lot 1), CYP2A2 (lot 1), CYP2B1 (lot 2), CYP2C6 (lot 1), CYP2C13 (lot 1), CYP2D1 (lot 2), CYP3A1 (lot 1) and CYP3A2 (lot 1) were used. The reactions were carried out as described for the rat liver microsomal study. To examine the role of individual CYP isoforms involved in the metabolism of MPA, each of the recombinant CYPs (30 pmol of CYP/mL) described above was incubated with 0.25 μM MPA for 30 min at 37 °C, according to the procedure recommended by the supplier.

Other microsomal enzyme activities

The activities of 7-ethoxyresorufin *O*-deethylase (EROD) and 7-benzyloxyresorufin *O*-debenzylase (BROD) were determined by an HPLC-fluorescence method. 7-Ethoxyresorufin (5 μM) or 7-benzylox-

yresorufin (5 μM) was incubated with 0.05 mg protein/mL of microsomes at 37 °C for 5 min as described for the assay of MPA metabolism in rat liver microsomes. 7-Ethoxyresorufin and 7-benzyloxyresorufin were dissolved in dimethylsulfoxide and added to the incubation mixture at final dimethylsulfoxide concentrations of 1% and 0.5%, respectively. After reaction had been stopped by adding 100 μL of cold acetonitrile, 50 or 20 μL of methoxyresorufin (20 mM in dimethylsulfoxide) was added as an internal standard for EROD and BROD activities, respectively. The mobile phase consisited of 20 mM potassium sodium phosphate buffer (pH 7.4)/acetonitrile (45/55, v/v) containing 2.5 mM of tetra-*n*-octylammonium bromide with a flow rate of 1.0 mL/min. The eluent was monitored at an excitation wavelength of 530 nm and emission wavelength of 580 nm by using a model 821-FP fluorescence detector (Jasco, Tokyo, Japan). Testosterone (50 μM) was incubated with 0.05–0.1 mg protein/mL of microsomes for 7–15 min. Formed 6β-hydroxytestosterone was measured as described previously (Kobayashi et al., 2000).

CYP2C contents of rat liver microsomes were determined by SDS-polyacrylamide gel electrophoresis and immunoblotting as described by Laemmli (1970) and Guengerich et al. (1982), respectively. Goat anti-rat CYP2C11 antiserum (Daiichi Pure Chemicals) was used, and the immunoblots were developed using a VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) and 3, 3'-diaminobenzidine. Anti-rat CYP2C11 antibodies reacted with CYP2C11, CYP2C6 and CYP2C13. Therefore, the CYPs that cross-reacted with anti-rat CYP2C11 antibodies are referred to as CYP2C. The intensities of the immunoblots were measured using a model GT-9600 scanner (EPSON, Tokyo, Japan) equipped with NIH Image/Gel Analysis Program (Ver. 1.61) adapted for Macintosh computers.

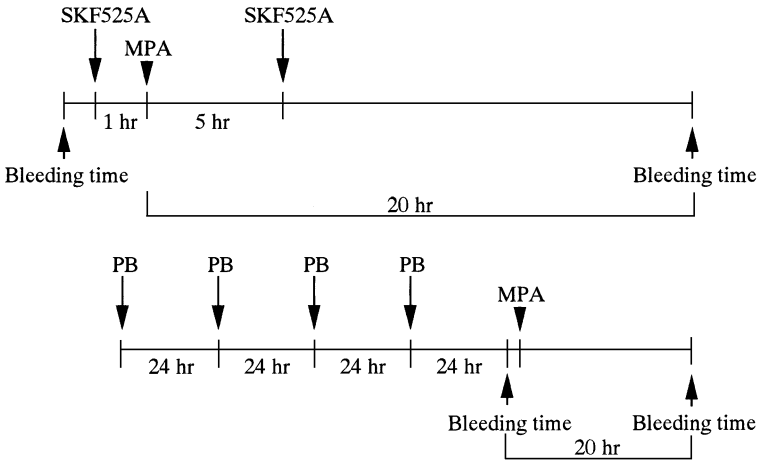
Effect of MPA on bleeding time

The bleeding time was measured by the method of Nobukata et al. (1999) with slight modification. In brief, animals anesthetized with diethylether were placed in a cage at a room temperature of 25—30 °C with their tails hanging down vertically. At the ventral part of the tail of each animal, an incision (5 mm long and 1 mm deep) was made without injuring the large vein or artery. Blood was carefully blotted every 15 s onto filter paper until the bleeding had stopped. To avoid circadian fluctuations in the bleeding time, all measurements were carried out between 10 and 12 in the morning. The bleeding time was measured before treatment and at 20 hr after treatment with MPA (100 mg/kg dissolved in 0.5% carboxymethyl cellulose, po). Changes from the bleeding time before dosing were calculated.

Effects of SKF525A and PB on change in bleeding time by MPA

To examine the effects of CYP modulators (i.e., inhibitor or inducer) on change in bleeding time after a single po administration of MPA, animals were pretreated with SKF525A (inhibitor of CYPs), PB (inducer of CYPs) or the vehicle alone.

The animals were divided into four groups (SKF525A-treatment group, control group for SKF525A, PB-treatment group and control group for PB). Rats were treated with SKF525A (n = 5, 100 mg/kg dissolved in saline, ip) or saline (n = 4, ip) 1 hr before and 5 hr after MPA treatment. Rats were treated with PB (n = 5, 100 mg/kg/day in saline, ip) or saline (n = 4, ip) for 4 days followed by MPA administered 24 hr after the last injection of PB (Scheme 1).



Scheme 1. Time-schedule for administration of drugs and measurement of bleeding time. The animals were divided into four groups (SKF525A-treatment group, control group for SKF525A, PB-treatment group and control group for PB). Rats were treated by po administration for MPA and ip administration for SKF525A and PB.

Statistical analyses

Results are expressed as means ± SD throughout the text. Student's *t*-test was used to analyze the significance of differences. A difference was regarded as statistically significant at the $p < 0.05$ level.

Results

CYP-dependent disappearance of MPA

When MPA (0.25 μM) was incubated with liver microsomes (0.1 mg protein/mL) prepared from an untreated rat for 15 min at 37 °C, disappearance of MPA in rat liver microsomes was found to be dependent on NADPH and was completely inhibited by SKF525A (1 mM), a typical CYP inhibitor (data not shown). These results suggest that the disappearance of MPA in rat liver microsomes is a CYP-dependent metabolic process.

Next, effects of chemical inhibitors of CYPs on the disappearance of MPA in liver microsomes prepared from an untreated rat were examined. Ketoconazole inhibited the disappearance of MPA in a concentration-dependent manner. The disappearance of MPA was inhibited to 34 and 3% of the control by 0.1 and 1 μM ketoconazole, respectively. Furaphylline (10 μM) inhibited the disappearance of MPA to 45% of the control, although the extent of inhibition was slight at a concentration of 1 μM (<25%). The inhibitory effect of sulfaphenazole on the disappearance of MPA was weak (<25%), even at a concentration of 10 μM.

Effects of CYP-inducers on intrinsic clearance of MPA

The intrinsic clearance of MPA in microsomes of a DEX-treated rat was 18-fold higher than that in microsomes of corn oil-treated rats (3.3 vs. 0.18 ± 0.04 mL/min/mg protein). The intrinsic clearance of

MPA in microsomes of PB-treated rats was 8-fold higher than that in microsomes of saline-treated rats (1.4 ± 0.26 vs. 0.15 ± 0.02 mL/min/mg protein, $p < 0.05$). There was no difference between the intrinsic clearance of MPA in microsomes of BNF-treated rats and in microsomes of corn oil-treated rats (0.24 ± 0.03 vs. 0.18 ± 0.04 mL/min/mg protein). As shown in Fig. 1, the intrinsic clearance of MPA in liver microsomes of rats treated with various CYP-inducers showed a significant correlation with testosterone 6β-hydroxylase activity ($r = 0.921$, $p < 0.001$). No other significant correlation was observed between the intrinsic clearance of MPA and EROD activity ($r = 0.270$), BROD activity ($r = 0.441$) or CYP2C contents ($r = 0.185$).

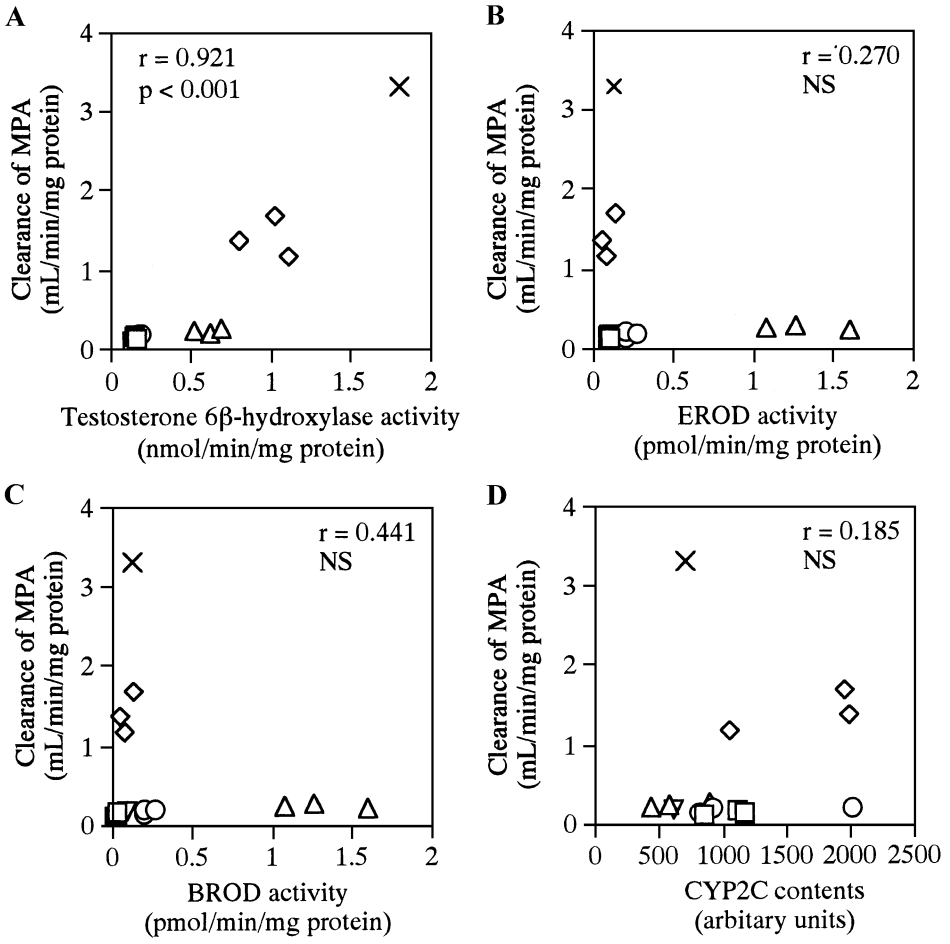


Fig. 1. Correlation of clearance of MPA with testosterone 6β-hydroxylase (A), EROD (B) and BROD activities (C) and CYP2C contents (D) in female rat liver microsomes. Microsomes were obtained from an untreated rat (▽, n = 1), and from rats treated with corn oil (○, n = 3), saline (□, n = 3), BNF (△, n = 3), PB (◇, n = 3) and DEX (×, n = 1). The protein concentration used for determining the clearance of MPA was 0.1 mg/mL in all cases except for 0.01 and 0.025 mg/mL for DEX- and PB-treated rats, respectively. Testosterone (50 μM) was incubated with 0.1 mg protein/mL of rat liver microsomes for 15 min. 7-Ethoxyresorufin (5 μM) and 7-benzyloxyresorufin (5 μM) were incubated with 0.05 mg protein/mL of rat liver microsomes for 5 min.

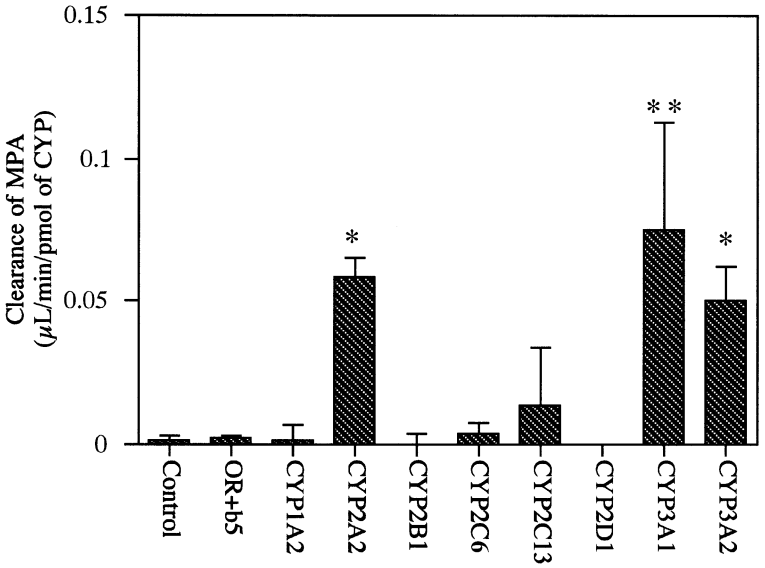


Fig. 2. Clearance of MPA in microsomes from baculovirus-infected insect cells expressing rat CYP1A2, CYP2A2, CYP2B1, CYP2C6, CYP2C13, CYP2D1, CYP3A1 and CYP3A2. MPA (0.25 μM) was incubated with microsomes (30 pmol of CYP/mL) at 37 °C for 30 min. Data are estimated from elimination rate constant of MPA in the incubation medium. Each column represents the mean ± SD of triplicate experiments. *$p < 0.05$; **$p < 0.01$ compared to microsomes expressing OR and b_5.

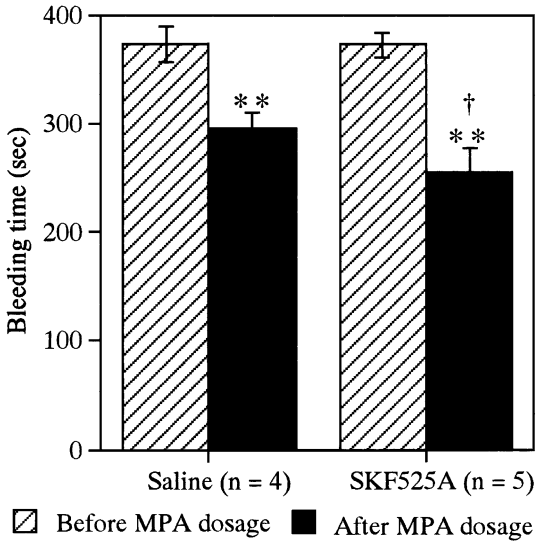


Fig. 3. Bleeding time before treatment (hatched bars) and at 20 hr after a single po administration of MPA (solid bars) in rats pretreated with saline or SKF525A. Rats were treated with MPA and SKF525A as described in Scheme 1. Each column represents the mean ± SD of data obtained from four or five rats. ** $p < 0.01$ compared with before MPA treatment, † $p < 0.05$ compared with the saline-treated group.

Study using recombinant rat CYPs

Microsomes from baculovirus-infected insect cells expressing CYP1A2, CYP2A2, CYP2B1, CYP2C6, CYP2C13, CYP2D1, CYP3A1 or CYP3A2 were examined in terms of the abilities of individual CYP isoforms to catalyze the metabolism of MPA (Fig. 2). Of the recombinant rat CYPs studied, CYP2A2, CYP3A1 and CYP3A2 showed remarkable disappearance of MPA by incubation. The clearance of MPA after incubation with CYP2A2, CYP3A1 and CYP3A2 were 0.059, 0.075 and 0.050 μL/min/pmol of CYP, respectively. CYP2C13 slightly showed the disappearance of MPA by incubation (0.013 μL/min/pmol of CYP). Control microsomes and the other recombinant CYPs exhibited no significant activity.

Effect of SKF525A on change in bleeding time by MPA treatment

As shown in Fig. 3, MPA significantly shortened the bleeding time at 24 hr after a single po administration in both the groups of saline-treated (373 ± 17 to 296 ± 14 sec, $p < 0.01$) and SKF525A-treated rats (372 ± 11 to 255 ± 23 sec, $p < 0.01$). The shortening effect of MPA on the bleeding time in the SKF525A-treated group was significantly stronger than that in the saline-treated group (-117 ± 20 vs. -77 ± 7 sec, $p < 0.05$).

Effect of PB on change in bleeding time by MPA treatment

As shown in Fig. 4, MPA significantly shortened the bleeding time at 24 hr after a single po administration in rats pretreated with saline (426 ± 20 to 351 ± 36 sec, $p < 0.01$). On the other hand, in the rats pretreated with PB, MPA did not cause any significant change in the bleeding time (395 ± 31 to 393 ± 39 sec).

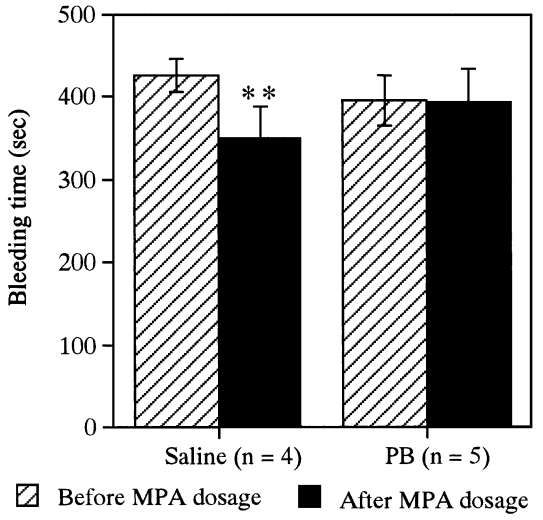


Fig. 4. Bleeding time before treatment (hatched bars) and at 20 hr after a single po administration of MPA (solid bars) in rats pretreated with saline or PB. Rats were treated with MPA and PB as described in Scheme 1. Each column represents the mean ± SD of data obtained from four or five rats. ** $p < 0.01$ compared with that before MPA treatment.

Discussion

The results of the present study suggest that CYP3A is the principal enzyme responsible for the CYP-catalyzed metabolism of MPA in liver microsomes of female rats. The supporting evidence can be summarized as follows. First, intrinsic clearance of MPA in liver microsomes of female rats was induced by DEX and PB, CYP3A inducers (Fig. 1). Second, the intrinsic clearance of MPA in liver microsomes of female rats treated with various CYP-inducers was highly correlated with testosterone 6β-hydroxylase activity (Fig. 1). Third, ketoconazole (1 μM), an inhibitor of CYP3A, potently inhibited the disappearance of MPA in rat liver microsomes. Fourth, a significant disappearance of MPA was observed in cDNA-expressed CYP3A1 and CYP3A2 (Fig. 2).

In the present study, the disappearance of MPA in rat liver microsomes was inhibited by furaphylline (an inhibitor of CYP1A) and sulfaphenazole (an inhibitor of CYP2C) to 45% and 25% of the control, respectively. However, intrinsic clearance of MPA in liver microsomes of female rats was not induced by BNF, a potent CYP1A inducer (Fig. 1). In addition, no disappearance of MPA was observed in cDNA-expressed CYP1A2 (Fig. 2). Therefore, the contribution of CYP1A to the overall metabolism of MPA in rat liver microsomes appears to be negligible. On the other hand, slight disappearance of MPA was observed in cDNA-expressed CYP2C13 (Fig. 2), whereas the intrinsic clearance of MPA in liver microsomes of female rats treated with various CYP-inducers was not correlated with CYP2C contents (Fig. 1). The results suggest that CYP2C might be involved in the CYP-catalyzed metabolism of MPA in rat liver microsomes as minor enzymes.

The present study using cDNA-expressed CYPs showed that a remarkable disappearance of MPA occurred not only in the presence of CYP3A but also in the presence of CYP2A2 (Fig. 2). This finding indicates that MPA is a substrate of these isoforms. On the other hand, it is well known that the expression of the constitutive forms in rats depends on sex (Pampori and Shapiro, 1999). Since CYP3A2 and CYP2A2 are male-specific isoforms, these isoforms are not likely to be principal enzymes responsible for the CYP-catalyzed metabolism of MPA in liver microsomes of female rats.

Although PB is a typical CYP2B inducer, no increase of BROD activity in microsomes was observed after PB treatment in the present study. At the present time, we have no idea because of limited induction studies using female rats. However, Yoshinari et al. (2001) reported that PB induced CYP2B1 mRNA in male WKY (Wistar Kyoto) rats but not female rats. Induction of *CYP2B1* gene by PB might be also sexually dimorphic in Wistar rats.

SKF525A is well known as a typical inhibitor of CYP. Saarni et al. (1983) reported that SKF525A administered with MPA caused a smaller decrease in the plasma concentration of MPA compared with that in the case of administration of MPA alone in rats. In this study, oral administration of MPA significantly shortened the bleeding time in both the groups of saline-treated and SKF525A-treated rats. The shortening effect of MPA on bleeding time in the SKF525A-treated group was significantly stronger than that in the saline-treated group (Fig. 3). These results suggest that metabolism of MPA mainly catalyzed by CYP3A1 in female rats was inhibited by SKF525A and that the metabolic inhibition of CYP3A1 by SKF525A caused an increase in plasma concentration of MPA, which resulted in enhancement of MPA-induced hypercoagulation.

Although oral administration of MPA significantly shortened the bleeding time in saline-treated rats, MPA did not cause any significant change in the bleeding time in PB-treated rats (Fig. 4). As shown in Fig. 1, the intrinsic clearance of MPA in microsomes of PB-treated rats was higher than that in the control rats. In addition, PB administered with MPA caused a greater decrease in the plasma concentration of

MPA compared with that in the case of administration of MPA alone in rats (Saarni et al., 1983). These findings suggest that metabolism of MPA mainly catalyzed by CYP3A1 in female rats was induced by PB and that the metabolic induction of CYP3A1 by PB caused a decrease in the plasma concentration of MPA, which resulted in reduction of MPA-induced hypercoagulation.

Although the results of the present study suggested that MPA itself but not CYP-dependent metabolite(s) is responsible for MPA-induced hypercoagulation, it is possible that MPA metabolite(s) formed via non-CYP enzyme is responsible for the blood coagulation in vivo. This is because bioavailability of orally administered MPA (i.e., via intestinal CYP3A) may be influenced by SKF525A and PB. However, first metabolic process of MPA appears to be mainly dependent on CYP, since intraperitoneally administered ^{3}H-MPA was rapidly changed to polar metabolites in PB-treated rats, while the production of these metabolites decreased in SKF525A-treated rats (Saarni et al., 1983). Therefore, we assumed that MPA itself rather than MPA metabolites is a predominant factor being responsible for the effects of MPA administration on bleeding time in vivo and its effect was influenced by CYP-dependent metabolism of MPA.

Conclusion

The results of the present study using in vitro and in vivo techniques suggested that the CYP-catalyzed metabolism of MPA is mainly catalyzed by CYP3A1 in liver microsomes of female rats and that the MPA-induced hypercoagulation in female rats is induced by MPA itself. Since MPA is metabolized by CYP3A4 in humans (Kobayashi et al., 2000), decreased capacity of CYP3A4 may be one of the factors causing an increase in the plasma concentration of MPA itself, which results in MPA-induced thrombosis.

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