

# Effect of oral administration of clinically relevant doses of dexamethasone on regulation of cytochrome P450 subfamilies in hepatic microsomes from dogs and rats

Kewei Zhang, DVM; Masanori Kuroha, DVM, PhD; Yuko Shibata, DVM; Eiichi Kokue, DVM, PhD; Minoru Shimoda, DVM, PhD

**Objective**—To evaluate the effect of oral administration of dexamethasone (DEX) at clinically relevant doses on metabolic activities of cytochrome P450 (CYP) isoenzymes in dogs and rats.

**Animals**—15 healthy 1-year-old male Beagles and 20 healthy 10-week-old male Wistar rats.

**Procedure**—Hepatic microsomes were harvested from dogs treated orally with DEX at 2.5 and 7.5 mg for 5 days and from rats treated orally with DEX at 0.75, 6, and 48 mg/kg for 5 days. 7-ethoxyresorufin, tolbutamide, bufuralol, and midazolam were used as CYP1A, CYP2C, CYP2D, and CYP3A substrates, respectively. Concentrations of metabolites formed by CYPs were measured by use of high-performance liquid chromatography, except for the resorufin concentrations measured by use of a fluorometric method. Reaction velocity-substrate concentration data were analyzed to obtain maximum reaction velocity ( $V_{max}$ ) and Michaelis-Menten constant ( $K_m$ ).

**Results**—Values of  $V_{max}$  for midazolam 4-hydroxylation were significantly decreased by treatment with DEX at 2.5 and 7.5 mg in dogs, although values of  $K_m$  were not affected. Values of  $V_{max}$  for bufuralol 1'-hydroxylation were also decreased by treatment with DEX. In rats, values of  $V_{max}$  for midazolam 4-hydroxylation were significantly decreased by treatment with DEX at 0.75 and 6 mg/kg but significantly increased at 48 mg/kg. Other reactions were not affected by treatment with DEX.

**Conclusions and Clinical Relevance**—Our results indicate that DEX downregulates the CYP3A subfamily when administered at clinically relevant doses to dogs. The effect of downregulation of CYP3A in dogs treated with DEX should be considered to avoid adverse effects from coadministration of drugs. (*Am J Vet Res* 2006;67:329–334)

Glucocorticoids are widely used to treat many conditions, including skin disease, eye disease, arthritis, acute inflammation caused by trauma, adrenocortical collapse, hypersensitivity reactions, and stress, and to provide immunosuppressive therapy. Although many glucocorticoids are available, the use of DEX is a common choice in veterinary medicine as well as human medicine because it lacks a sodium and water

retention effect and has a high potency and long half-life.

It is well known that DEX is a potent inducer of the CYP3A subfamily (ie, CYP3A). This has been demonstrated in many animal species, such as humans,<sup>1</sup> rats,<sup>2,3</sup> miniature pigs,<sup>4</sup> and monkeys,<sup>5</sup> by use of in vitro or in vivo experiments. Coadministration of DEX with other drugs is a frequent practice in veterinary medicine. Dexamethasone administration may therefore result in an undesirable drug-drug interaction in clinically affected individuals by the induction of CYP3A activity if drugs that are administered together are metabolized by CYP3A. Yule et al<sup>6</sup> have reported that cyclophosphamide pharmacokinetics was affected by coadministration with DEX in children with cancer. Because many drugs are metabolized by CYP3A, the potential for a drug-drug interaction to occur may be substantial in treatments that include DEX administration.

Because drugs generally have dose-related effects, the effect of enzyme induction by DEX may also be dose dependent. Information about the induction of CYP3A by DEX administration at clinically relevant doses may therefore be important. However, to our knowledge, few reports exist on the effect of enzyme induction by DEX when used at clinically relevant doses. In some studies on CYP3A induction, DEX has been administered at > 50 mg/kg. In studies by Eeckhoudt et al<sup>2</sup> and Meredith et al,<sup>3</sup> the effect of enzyme induction by DEX was examined in rats when given at 50 mg/kg. Ojha and Kohli<sup>5</sup> have demonstrated a substantial effect of enzyme induction by DEX in monkeys when given at 150 mg/kg. These doses are higher than those used in small animal medicine (ie, 0.1 to 6 mg/kg).<sup>7</sup> The purpose of the study reported here was to examine the effect of oral administration of DEX on CYP3A activities in dogs when DEX is given at clinically relevant amounts. We also examined the effect of DEX administration on activities of other CYPs that are related to drug metabolism, such as CYP1A, CYP2C, and CYP2D subfamilies. Because DEX is a substrate for the CYP3A subfamily, its possible inhibitory effects on CYP3A activities were also examined by in vitro experiments to elucidate the

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From the Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan.

Address correspondence to Dr. Shimoda.

DEX	Dexamethasone
CYP	Cytochrome P450
$K_m$	Michaelis-Menten constant
CV	Coefficient of variation
HPLC	High-performance liquid chromatography
$V_{max}$	Maximal reaction velocity
$K_i$	Inhibitory constant

inhibitory mode (competitive or noncompetitive) and calculate the inhibitory constant.

## Materials and Methods

**Effect of oral administration of DEX**—Fifteen male Beagles<sup>a</sup> (age, 1 year; body weight, 11 to 15 kg) were randomly assigned to 3 groups of 5 dogs each. Dogs were kept in individual cages, allowed access to water ad libitum, and given food twice a day (8:00 AM and 8:00 PM). Dogs from 2 groups were given DEX tablets<sup>b</sup> orally once a day between meals (2:00 PM) for 5 days at a dose of 2.5 mg (0.17 to 0.23 mg/kg) or 7.5 mg (0.5 to 0.68 mg/kg), respectively. Dogs from the third group served as controls. At 24 hours after administration of the final dose, dogs were deeply anesthetized by IV injection of sodium pentobarbital (25 mg/kg) and exsanguinated from a cannula inserted into the carotid and specimens of liver tissue were collected. Liver specimens were stored at  $-80^{\circ}\text{C}$  until preparation of hepatic microsomes.

We also examined the effect of oral administration of DEX on CYP activities in rats. Twenty male Wistar rats<sup>c</sup> (age, 10 weeks; body weight, 230 to 300 g) were assigned to 4 groups of 5 rats each. Rats from 3 groups were given DEX<sup>d</sup> (suspended in 0.5% carboxymethylcellulose) orally once a day (2:00 PM) for 5 days at 0.75, 6, and 48 mg/kg, respectively. Rats from the fourth group were given 0.5% carboxymethylcellulose orally and served as controls. At 24 hours after the final dose administration, rats were deeply anesthetized by inhalation with ether and exsanguinated from the abdominal aorta by use of a needle and syringe and specimens of liver tissue were collected. Experiments were conducted in accordance with guidelines for the care and use of laboratory animals, Faculty of Agriculture, Tokyo University of Agriculture and Technology.

**Inhibition studies**—Reversible and irreversible inhibition for CYP3A activities was examined by use of canine microsomes obtained from control dogs. In the study of reversible inhibition, DEX in methanol solution was mixed with substrate (midazolam) solutions at various concentrations and then added to the assay system (reaction mixture), which yielded 80  $\mu\text{g/mL}$  (204 $\mu\text{M}$ ) of DEX and 7.67 to 230.2 $\mu\text{M}$  of midazolam as final concentrations. In the study of irreversible inhibition, the DEX solution (80  $\mu\text{g/mL}$ ) was added at 15 or 30 minutes before the substrate solution (60  $\mu\text{M}$ ) was added.

**Preparation of hepatic microsomes**—Microsomal fractions were prepared from the liver specimens as described by Van der Hoeven and Coon.<sup>8</sup> Obtained liver specimens were frozen at  $-80^{\circ}\text{C}$  until used. Protein concentrations and CYP contents in liver specimens were determined as described by Lowry et al<sup>9</sup> and Omura and Sato,<sup>10</sup> respectively.

**Enzyme-specific assays**—Enzyme kinetics of the CYP1A, CYP2C, CYP2D, and CYP3A subfamilies were examined by use of 7-ethoxyresorfin O-deethylation, tolbutamide hydroxylation, bufuralol 1'-hydroxylation, and midazolam 4-hydroxylation, respectively, although it is not fully known whether the CYP subfamilies catalyze these reactions in dogs. The reaction proceeded at  $37^{\circ}\text{C}$  in a reaction mixture that contained a system of NADPH generation (50mM phosphate buffer [pH, 7.4], 0.5mM NADP<sup>+</sup>, 5mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase [1.5 U/mL], and 5mM  $\text{MgCl}_2$ ), liver microsomes (approx 0.5 mg/mL), and a substrate at various concentrations in a total volume of 250  $\mu\text{L}$ , except for ethoxyresorfin O-deethylation, which was conducted in a total volume of 1 mL. A 5-minute preincubation step at  $37^{\circ}\text{C}$  was performed before the reaction was started by the addition of substrate.

**Determination of CYP1A activity**—The metabolite of 7-ethoxyresorfin, resorufin,<sup>e</sup> was measured by use of a fluorometric method described previously.<sup>11</sup> For the present study on dogs, concentrations of 7-ethoxyresorfin<sup>f</sup> in the assay system ranged from 0.065 to 2.07 $\mu\text{M}$ . For the present study on rats, the concentration of the substrate in the assay system was 2 $\mu\text{M}$ , which is approximately 10-fold higher than the concentration of the  $K_m$  (0.227 $\mu\text{M}$ ) reported for rats by Hanioka et al.<sup>12</sup> Reactions were terminated by the addition of 3 mL of methanol at 15 minutes after 7-ethoxyresorfin was added, followed by placement on ice for 5 minutes. After centrifugation at 2,000  $\times$  g for 5 minutes, 1 mL of the resulting supernatant was transferred to a clear test tube, diluted with 4 mL of methanol, and then applied to a spectrofluorometer.<sup>8</sup> Excitation and emission wavelengths were set at 550 and 586 nm, respectively. The recovery of resorufin was  $103 \pm 6\%$  (CV, 5.9%) at 20nM (n = 4). The intraday CV was 3.7% at 20nM (n = 4). Interday CVs ranged from 3.4% to 6.4% at 20nM (3 days, 4 determinations/d).

**Determination of CYP2C activity**—The metabolite of tolbutamide, hydroxytolbutamide,<sup>h</sup> was measured by use of HPLC, as previously reported.<sup>13</sup> For the study on dogs, concentrations of tolbutamide<sup>i</sup> in the assay system ranged from 1 to 8mM. For the study on rats, the concentration of tolbutamide in the assay system was 10mM, which is approximately 5-fold higher than the  $K_m$  (1.91mM) reported for rats by Easterbrook et al.<sup>14</sup> After the addition of tolbutamide, the solution was incubated for 30 minutes and reactions were quenched by 0.15M phosphoric acid (250  $\mu\text{L}$ ). Thirty microliters of chlorpropamide<sup>j</sup> solution (10  $\mu\text{g/mL}$ ) was added as an internal standard. Samples were mixed with 3 mL of diethyl ether. After centrifugation at 2,000  $\times$  g for 5 minutes, the upper organic layer was transferred to a clean pear-shaped flask. The solvent was evaporated to dryness under reduced pressure. The residue was reconstituted with 250  $\mu\text{L}$  of mobile phase, and 100  $\mu\text{L}$  of solution was injected into a reversed-phase column.<sup>k</sup> The column effluent was monitored by UV absorbance at 230 nm. The gradient-elution program was used to detect hydroxytolbutamide and chlorpropamide. Solvent A was 50mM phosphate buffer (pH, 4.3), and solvent B was acetonitrile. The solvent composition was held at 75% solvent A for 8 minutes and then changed linearly to 80% solvent B by 15 minutes. The flow rate was 1.0 mL/min. Recoveries of hydroxytolbutamide and chlorpropamide were  $91.2 \pm 1.2\%$  (CV, 1.3%) and  $96.2 \pm 3.7\%$  (CV, 3.9%) at 1 and 10  $\mu\text{g/mL}$ , respectively (n = 4). Interday CVs in the assay were 1.3% to 6.3% at 1  $\mu\text{g/mL}$  (3 days; 4 determinations/d), with a limit of quantification of 5 ng/mL at a signal-to-noise ratio of 3.

**Determination of CYP2D activities**—The metabolite of bufuralol, 1'-hydroxybufuralol,<sup>l</sup> was measured by use of HPLC, as described previously.<sup>15</sup> For the present study on dogs, concentrations of bufuralol<sup>m</sup> in the assay system ranged from 6.25 to 200mM. For the study on rats, the concentration of bufuralol in the assay system was 103.6mM, which is 10-fold higher than the  $K_m$  (6.4 $\mu\text{M}$ ) reported by Chow et al.<sup>16</sup> After the addition of bufuralol, the solution was incubated for 10 minutes and reactions were stopped by the addition of 30  $\mu\text{L}$  of 60% perchloric acid. The denatured protein was precipitated by centrifugation at 10,000  $\times$  g for 2 minutes, and 20  $\mu\text{L}$  of the supernatant was applied to a reversed-phase column.<sup>k</sup> The mobile phase consisted of 10mM perchloric acid and acetonitrile (65:35 [vol/vol]). The flow rate was 1.0 mL/min. Excitation and emission wavelengths for fluorometric determination of 1'-hydroxybufuralol were 252 and 302 nm, respectively. The recovery of 1'-hydroxybufuralol was  $91.4 \pm 3.0\%$  (CV, 3.3%) at 0.1  $\mu\text{g/mL}$  (n = 5). Interday CVs were 1.9% to 7.9% at 0.1  $\mu\text{g/mL}$  (3 days; 4 determina-

Table 1—Mean  $\pm$  SD total hepatic microsome CYP content from 15 dogs and 20 rats treated orally with DEX once a day for 5 days.

Dogs (n = 5/treatment)		Rats (5/treatment)	
DEX dose (mg)	CYP (nmol/mg protein)	DEX dose (mg/kg)	CYP (nmol/mg protein)
0 (control)	0.860 $\pm$ 0.251	0 (control)	1.83 $\pm$ 0.18
2.5	0.601 $\pm$ 0.093	0.75	0.861 $\pm$ 0.248*
7.5	0.483 $\pm$ 0.078*	6	1.21 $\pm$ 0.27*
		48	2.47 $\pm$ 0.21*

\*Significantly ( $P < 0.05$ ) different from control value in this species.

tions/d), with a limit of quantification of 5 ng/mL at a signal-to-noise ratio of 3.

**Determination of CYP3A activities**—The metabolite of midazolam, 4-hydroxymidazolam,<sup>n</sup> was determined by use of HPLC, as described previously.<sup>17</sup> Midazolam concentrations in the assay system ranged from 15.3 to 307  $\mu$ M for the study on dogs and from 30.7 to 307  $\mu$ M for the study on rats. After the addition of midazolam, the mixture was incubated for 10 minutes and reactions were quenched by the addition of 250  $\mu$ L of acetonitrile and placement on ice. After centrifugation at 10,000  $\times$  g for 2 minutes, the resulting supernatant was applied to a reversed-phase column.<sup>k</sup> The mobile phase consisted of 100mM acetate buffer (pH, 4.7), acetonitrile, and methanol (53:41.4:5.6 [vol/vol/vol]). Column effluent was monitored by UV absorbance at 254 nm. The recovery of 4-hydroxymidazolam was 101  $\pm$  6% at 1  $\mu$ g/mL (n = 4). Interday CVs were 3.7% to 7.6% at 1  $\mu$ g/mL (3 days; 4 determinations/d). The quantification limit was 2.5 ng/mL at a signal-to-noise ratio of 3.

**Enzyme kinetic analysis**—The metabolism of 7-ethoxyresorufin, tolbutamide, bufuralol, and midazolam in liver microsomes was consistent with single-enzyme Michaelis-Menten kinetics, as reported by Kuroha et al.<sup>18</sup> Accordingly, the following equation was used to describe the relation between reaction velocities (ie, V) and substrate concentrations (ie, S):

$$V = \frac{V_{\max} \times S}{K_m + S}$$

By use of a nonlinear least squares fitting program,<sup>19</sup> the  $V_{\max}$  and  $K_m$  values were calculated.

In the inhibition study, the relation between reaction velocities of midazolam 4-hydroxylation and midazolam concentrations was expressed by use of the following equation:

$$V = \frac{V_{\max} \times S}{K_m \left(1 + \frac{I}{K_i}\right) + S}$$

where I is the concentration of inhibitor (ie, DEX). Two curves, which were obtained with or without DEX, were simultaneously analyzed by use of the fitting program to calculate  $V_{\max}$ ,  $K_m$ , and the  $K_i$ .

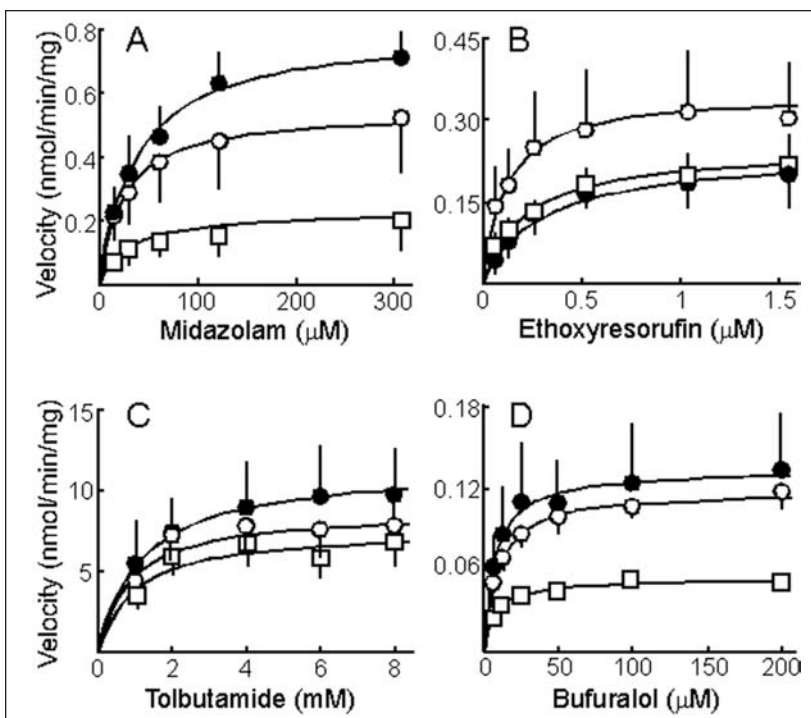


Figure 1—Mean  $\pm$  SD (n = 5) Michaelis-Menten kinetic values of midazolam 4-hydroxylation (A), ethoxyresorufin O-deethylation (B), tolbutamide hydroxylation (C), and bufuralol 1'-hydroxylation (D) in hepatic microsomes from dogs treated orally with DEX (0 mg [closed circles], 2.5 mg [open circles], and 7.5 mg [open squares], respectively) once a day for 5 days. Lines were calculated by nonlinear least squares regression analysis.

**Statistical analysis**—Data were analyzed with an ANOVA after the confirmation of equal variance of variables among groups by use of the Bartlett test. From results of the Scheffe multiple comparison test, values of  $P < 0.05$  were considered significant.

## Results

**Effects of DEX on CYP contents of hepatic microsomes**—Hepatic microsome CYP content significantly changed in dogs and rats in response to oral administration of DEX (Table 1). Hepatic microsome CYP content of dogs treated with 2.5 and 7.5 mg of DEX was approximately 70% and 50% of that of control dogs, respectively. Hepatic microsome CYP content of dogs treated with 7.5 mg of DEX was significantly lower than that of control dogs. By comparison, hepatic microsome CYP content of rats treated with DEX at 0.75 and 6 mg/kg was significantly lower than that of control rats; however, hepatic microsome CYP content of rats treated with DEX at 48 mg/kg was significantly higher than that of control rats.

Table 2—Mean  $\pm$  SD (n = 5) Michaelis-Menten kinetic parameters for several metabolic reactions catalyzed by CYP subfamilies in hepatic microsomes from dogs treated orally with DEX once a day for 5 days.

Reactions	$V_{max}$ (nmol/min/mg of protein)			$K_m$ ( $\mu$ M)		
	0 mg DEX	2.5 mg DEX	7.5 mg DEX	0 mg DEX	2.5 mg DEX	7.5 mg DEX
Midazolam 4-hydroxylation	0.803 $\pm$ 0.116	0.544 $\pm$ 0.166*	0.233 $\pm$ 0.113*	37.9 $\pm$ 22.0	24.7 $\pm$ 5.9	31.7 $\pm$ 10.7
Ethoxyresorufin O-deethylation	0.242 $\pm$ 0.061	0.352 $\pm$ 0.111*	0.249 $\pm$ 0.074*	0.303 $\pm$ 0.138	0.113 $\pm$ 0.038	0.126 $\pm$ 0.101
Tolbutamide hydroxylation	0.0116 $\pm$ 0.0035	0.00879 $\pm$ 0.00161	0.00762 $\pm$ 0.00122	1,180 $\pm$ 588	858 $\pm$ 238	966 $\pm$ 356
Bufuralol 1'-hydroxylation	0.134 $\pm$ 0.044	0.118 $\pm$ 0.013	0.0534 $\pm$ 0.0041*	7.67 $\pm$ 2.00	8.87 $\pm$ 1.85	6.86 $\pm$ 1.23

See Table 1 for key.

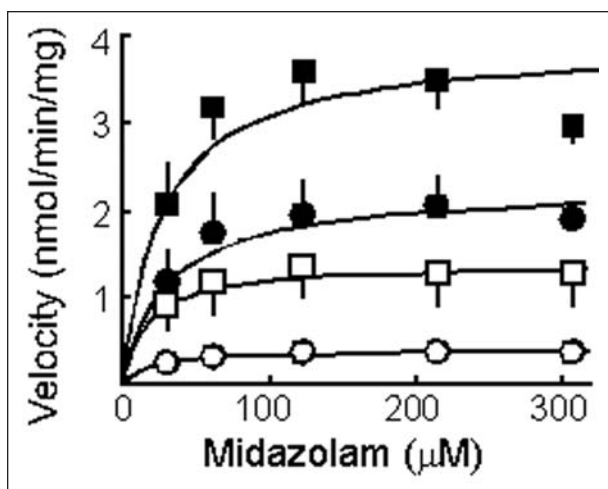


Figure 2—Mean  $\pm$  SD (n = 5) Michaelis-Menten kinetic values of midazolam 4-hydroxylation in hepatic microsomes from rats treated with DEX (0 mg/kg [closed circles], 0.75 mg/kg [open circles], 6 mg/kg [open squares], and 48 mg/kg [closed squares]) once a day for 5 days. Lines were calculated by the nonlinear least squares regression analysis.

**Effects of DEX on CYP3A activities**—Treatment with DEX with clinically relevant doses did not induce CYP3A activity in dogs (Figure 1). Reaction velocities of midazolam 4-hydroxylation were significantly decreased by treatment with DEX. Calculated values of  $V_{max}$  from dogs treated with 2.5 and 7.5 mg of DEX were 65% and 28%, respectively, of the  $V_{max}$  from control dogs. On the other hand, values of  $K_m$  in dogs were not affected by treatment with DEX (Table 2).

Treatment with DEX also affected CYP3A activities in rats (Figure 2). Reaction velocities were significantly decreased by treatment with DEX at 0.75 mg/kg, which is almost equivalent to a dose of 7.5 mg in dogs. At 6 mg/kg, however, reaction velocities in treated rats were similar to reaction velocities of control rats. At the highest dose (48 mg/kg), significant increases were found in reaction velocities. Values of  $V_{max}$  from rats treated with DEX at 0.75, 6, and 48 mg/kg were 27%, 93%, and 263%, respectively, of the  $V_{max}$  of control rats; the decrease in  $V_{max}$  for rats treated with DEX at 0.75 mg/kg and the increase in  $V_{max}$  for rats treated with DEX at 48 mg/kg were significantly different from values of  $V_{max}$  for control rats. Values of  $K_m$  in rats were also not affected by treatment with DEX (Table 3).

Table 3—Mean  $\pm$  SD (n = 5) Michaelis-Menten kinetic parameters for midazolam 4-hydroxylation in hepatic microsomes from rats treated orally with DEX once a day for 5 days.

DEX (mg/kg)	Midazolam 4-hydroxylation	
	$V_{max}$ (nmol/min/mg of protein)	$K_m$ ( $\mu$ M)
0	2.28 $\pm$ 0.34	29.6 $\pm$ 18.2
0.75	0.396 $\pm$ 0.157*	18.2 $\pm$ 5.4
6	1.142 $\pm$ 0.41	28.5 $\pm$ 7.1
48	3.82 $\pm$ 0.32*	31.5 $\pm$ 9.05

See Table 1 for key.

**Effects of DEX on CYP1A, CYP2C, and CYP2D activities**—Values of  $V_{max}$  for bufuralol 1'-hydroxylation (CYP2D activities) from dogs treated with DEX were lower than those from control dogs, and differences were significant between the dogs treated at 7.5 mg and control dogs (Table 1). On the other hand, values of  $V_{max}$  for ethoxyresorufin O-deethylation (CYP1A activities) and tolbutamide hydroxylation (CYP2C activities) in dogs were not affected by treatment, even at 7.5 mg. Values of  $K_m$  from each reaction were not significantly different between dogs treated with DEX and control dogs. In contrast to dogs, no significant changes were found for any reactions in rats (Table 4).

**Inhibitory effects of DEX on CYP3A activities in dogs**—Treatment with DEX inhibited midazolam 4-hydroxylation in a competitive manner (Figure 3). The mean  $\pm$  SD value for  $K_i$  was 104  $\pm$  16  $\mu$ M (n = 5). Reactions were not affected by pre-exposure to DEX for 15 and 30 minutes, which indicated that DEX did not cause irreversible inhibition of CYP3A activity (Figure 4).

## Discussion

Although DEX is a well-established inducer of CYP3A activity in many animal species,<sup>1-4</sup> few reports exist that describe the induction effects when the drug is administered at clinically relevant doses. We therefore examined the CYP3A induction effect of DEX in dogs by using clinically relevant doses and dosage intervals.<sup>7</sup> However, we found that CYP3A activities were unexpectedly lower in microsomes from dogs treated with DEX. Michaelis-Menten kinetic analysis indicated that the lower activities result from an alteration in values of  $V_{max}$  because values of  $K_m$  were not affected. These findings indicate that the lower activi-

Table 4—Mean  $\pm$  SD (n = 5) metabolic rates catalyzed by CYP1A, CYP2C, and CYP2D subfamilies in hepatic microsomes from rats treated orally with DEX once a day for 5 days.

Reactions*	Reaction velocity (nmol/min/mg of protein)			
	DEX (0 mg/kg)	DEX (0.75 mg/kg)	DEX (6 mg/kg)	DEX (48 mg/kg)
Ethoxyresorufin O-deethylation	1.11 $\pm$ 0.29	0.602 $\pm$ 0.275	0.914 $\pm$ 0.335	1.04 $\pm$ 0.17
Tolbutamide hydroxylation	0.772 $\pm$ 0.133	0.685 $\pm$ 0.273	0.580 $\pm$ 0.284	0.875 $\pm$ 0.197
Bufuralol 1'-hydroxylation	1.97 $\pm$ 0.28	1.76 $\pm$ 0.13	1.75 $\pm$ 0.39	1.89 $\pm$ 0.38

\*Substrate concentrations of ethoxyresorufin, tolbutamide, and bufuralol were 2, 10,000, and 103.6 $\mu$ M, respectively, and corresponded to values 5- to 10-fold higher than values of  $K_m$  of their metabolic reactions.

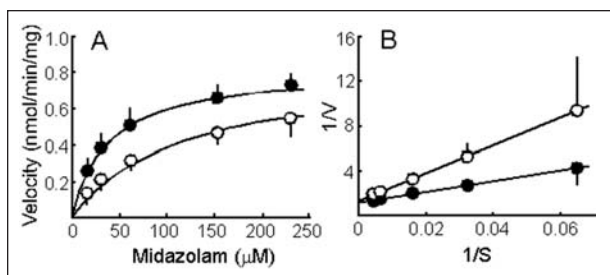


Figure 3—Mean  $\pm$  SD (n = 5) Michaelis-Menten kinetic values of midazolam 4-hydroxylation in canine hepatic microsomes (A) and the reciprocal plot (B) with the addition of 204 $\mu$ M DEX (open circles) or methanol (closed circles). V = Reaction velocities. S = Substrate concentrations.

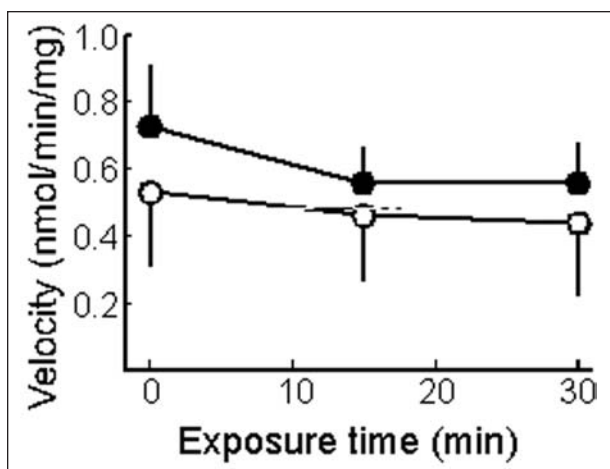


Figure 4—Mean  $\pm$  SD (n = 3) effect of exposure of canine hepatic microsomes to 204 $\mu$ M DEX (open circles) or methanol (closed circles) on midazolam 4-hydroxylation.

ties of CYP3A may be derived from a decrease in the amount of CYP3A, an effect of downregulation by DEX. Lower CYP contents in microsomes from dogs treated with DEX may support this suggestion.

As a causative factor of decreased activities of CYP3A, another possibility is that the administered DEX was present in the microsomes and inhibited midazolam 4-hydroxylation in the assay system, as DEX is a substrate for CYP3A. We therefore examined the inhibitory effect of DEX on midazolam 4-hydroxylation by using microsomes from the nontreated dogs. Dexamethasone competitively inhibited the reaction

with a  $K_i$  value of 104 $\mu$ M (approx 40  $\mu$ g/mL). The  $K_i$  value was too high to elicit substantial inhibition to CYP3A activities in the assay system. Concentrations of DEX in the assay system should have been much lower than the  $K_i$  value, considering the low dose of DEX administered (ie, 2.5 and 7.5 mg) and the microsome purification procedure. In addition, DEX was not found to be a mechanism-based inhibitor of CYP3A activity in our study.

Because the effect of downregulation of CYP3A by DEX might be species specific, we also examined the effect of downregulation in rats. The effect of downregulation was also observed when DEX was administered to rats at 0.75 mg/kg, which was almost equivalent to a dose of 7.5 mg in dogs. On the other hand, an induction effect of the drug in rats was observed when DEX was administered at 48 mg/kg, which was similar to doses used in many reports<sup>1-4</sup> that describe induction effects. Dexamethasone therefore had a dual effect on CYP3A activity in rats (ie, downregulation of CYP3A with low clinically relevant doses and upregulation with a much higher dose).

In addition to CYP3A activity, results of our study indicate that at clinically relevant doses of DEX, an effect of downregulation of CYP2D by DEX exists, but no effects exist on CYP1A and CYP2C activities in dogs. On the other hand, these CYPs were not affected in rats for any doses of DEX. Although no reports exist that describe an effect of downregulation of these CYPs by DEX, an effect of downregulation on CYP2E has been demonstrated by Kim et al.<sup>20</sup> Results of their western blot analysis revealed significantly lower expression of the enzyme in microsomes from rats after intraperitoneal injection of the drug at 5 mg/kg/d for 4 days. Their analysis also revealed lower expression of CYP2B, although CYP2B is induced by DEX at a high dose.<sup>3</sup> These findings may indicate that attention should be paid to the effect of downregulation, but not upregulation, of CYP subfamilies when patients are treated with DEX.

Although several reports<sup>21-23</sup> exist that describe the induction mechanisms of CYP3A activity by DEX, to our knowledge there are no reports that describe mechanisms by which DEX downregulates CYP3A. However, a mechanism of downregulation of CYP2B by DEX has been recently proposed by Ringel et al.<sup>24</sup> as follows: 1) expression of CYP2B requires formation of a

binding complex of constitutive androstane receptor, retinoid x receptor, DNA, and steroid receptor coactivator-1; 2) DEX binds to the constitutive androstane receptor and dissociates steroid receptor coactivator-1 from the binding complex; and 3) because of the dissociation, the transcription of CYP2B mRNA is inhibited, which results in a decrease in expression of CYP2B. Because the constitutive androstane receptor has been also recognized as a regulator for the expression of CYP3A, the effect of downregulation by DEX in our study may be derived from a similar mechanism, a possibility that should be examined in future studies.

In summary, results of our study indicate that DEX downregulates CYP3A at clinically relevant doses in dogs. The effect of downregulation may be cumulative because the decrease in CYP3A activities depends on catabolism of CYP3A. This suggests that DEX downregulates more potently if DEX is administered for a longer duration than that used in our study. We should therefore pay attention to a possible drug-drug interaction with CYP3A substrates that result from the effect of downregulation by DEX, especially in a long-term treatment. Unpredictable accumulation of CYP3A substrates may result in serious adverse effects in some circumstances. Oxidation of many drugs, including immunosuppressive drugs, antidepressants, calcium channel blockers, antimicrobials, vinca alkaloids, and others, is catalyzed by CYP3A. Therefore, the potential for the drug-drug interaction to occur may be substantial in treatment with DEX. This fact might be applied to animal species other than dogs because the effect of downregulation by DEX was also observed in rats. In addition, we should pay attention to a possible drug-drug interaction with CYP2D substrates in dogs because DEX also caused downregulation of the enzyme in our study.

- a. CSK Research Park Co Ltd, Nagano, Japan.
- b. Dexamethazone tablet, Asahi Kasei Corp, Osaka, Japan.
- c. Japan Clear, Tokyo, Japan.
- d. Dexamethazone, Wako Pure Chemical, Osaka, Japan.
- e. Resorufin, Sigma Chemical Co, St Louis, Mo.
- f. 7-Ethoxyresorufin, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.
- g. RF1500, Shimadzu Corp, Kyoto, Japan.
- h. Hydroxytolbutamide, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.
- i. Tolbutamide, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.
- j. Chlorpropamide, Sigma Chemical Co, St Louis, Mo.
- k. TSK-gel ODS-120T, 4.6 × 250 mm, TOSOH Co, Tokyo, Japan.
- l. Hydroxybufuralol, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.
- m. Bufuralol, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.
- n. 4-Hydroxymidazolam, Daiichi Pure Chemical Co Ltd, Tokyo, Japan.

## References

1. Williams JA, Chenery RJ, Hawksworth GM. Induction of CYP3A enzymes in human and rat hepatocyte cultures. *Biochem Soc Trans* 1994;22:131S.
2. Eeckhoudt SL, Horsmans Y, Verbeeck RK. Differential induction of midazolam metabolism in the small intestine and liver by oral and intravenous dexamethasone pretreatment in rat. *Xenobiotica* 2002;32:975-984.
3. Meredith C, Scott MP, Renwick AB, et al. Studies on the induction of rat hepatic CYP1A, CYP2B, CYP3A and CYP4A subfamily form mRNAs in vivo and in vitro using precision-cut rat liver slices. *Xenobiotica* 2003;33:511-527.

4. Lu C, Li AP. Species comparison in P450 induction: effects of dexamethasone, omeprazole, and rifampin on P450 isoforms 1A and 3A in primary cultured hepatocytes from man, Sprague-Dawley rat, minipig, and beagle dog. *Chem Biol Interact* 2001;134:271-281.

5. Ojha V, Kohli KK. Induction of hepatic microsomal cytochrome P450 by dexamethasone in rhesus monkey (*Macaca mulatta*). *Biochem Mol Biol Int* 1994;33:1135-1143.

6. Yule SM, Boddy AV, Cole M, et al. Cyclophosphamide pharmacokinetics in children. *Br J Pharmacol* 1996;41:13-19.

7. Boothe DM. Drug dosage tables. In: DM Boothe, ed. *Small animal clinical pharmacology and therapeutics*. Philadelphia: WB Saunders Co, 2001;733-770.

8. Van der Hoeven TA, Coon MJ. Preparation and properties of partially purified Cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase from rabbit liver microsomes. *J Biol Chem* 1974;249:6302-6310.

9. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-275.

10. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. *J Biol Chem* 1964;239:2370-2378.

11. Burke MD, Thompson S, Elcombe CR, et al. Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* 1985;34:3337-3345.

12. Hanioka N, Tatarazako N, Jinno H, et al. Determination of cytochrome P450 1A activities in mammalian liver microsomes by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 2000;744:399-406.

13. Wester MR, Lasker JM, Johnson EF, et al. CYP2C19 participates in tolbutamide hydroxylation by human liver microsomes. *Drug Metab Dispos* 2000;28:354-359.

14. Easterbrook J, Fackett D, Li AP. A comparison of aroclor 1254-induced and uninduced rat liver microsomes to human liver microsomes in phenytoin O-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, S-mephenytoin 4'-hydroxylation, chloroxazone 6-hydroxylation and testosterone 6beta-hydroxylation. *Chem Biol Interact* 2001;134:243-249.

15. Kronbach T. Bufuralol, dextromethorphan, and debrisoquine as prototype substrates for human P450IID6. *Methods Enzymol* 1991;206:509-517.

16. Chow T, Imaoka S, Hiroi T, et al. Developmental changes in the catalytic activity and expression of CYP2D isoforms in the rat liver. *Drug Metab Dispos* 1999;27:188-192.

17. Carrillo JA, Ramos SI, Agundez JA, et al. Analysis of midazolam and metabolites in plasma by high-performance liquid chromatography: probe of CYP3A. *Ther Drug Monit* 1998;20:319-324.

18. Kuroha M, Kuze Y, Shimoda M, et al. In vitro characterization of the inhibitory effects of ketoconazole on metabolic activities of cytochrome P-450 in canine hepatic microsomes. *Am J Vet Res* 2002;63:900-905.

19. Yamaoka K, Tanigawara Y, Nakagawa T, et al. A pharmacokinetic analysis program (MULTI) for microcomputer. *J Pharmacobiodyn* 1981;4:879-885.

20. Kim H, Putt DA, Zangar RC, et al. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metab Dispos* 2001;29:353-360.

21. Pascussi JM, Drocourt L, Fabre JM, et al. Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol* 2000;58:361-372.

22. Pascussi JM, Gerbal-Chaloin S, Fabre JM, et al. Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol Pharmacol* 2000;58:1441-1450.

23. Pascussi JM, Drocourt L, Gerbal-Chaloin S, et al. Dual effect of dexamethasone on CYP3A4 gene expression in human hepatocytes. Sequential role of glucocorticoid receptor and pregnane X receptor. *Eur J Biochem* 2001;268:6346-6358.

24. Ringel M, Oesch F, Gerl M, et al. Permissive and suppressive effects of dexamethasone on enzyme induction in hepatocyte co-cultures. *Xenobiotica* 2002;32:653-666.