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Toxicology

Characteristics of cytochrome P450-dependent metabolism in the liver of the wild raccoon, *Procyon lotor*

So SHINYA[1)], Yared Beyene YOHANNES[1)], Yoshinori IKENAKA[1–4)], Shouta MM NAKAYAMA[1,5)], Mayumi ISHIZUKA[1)]*, Shoichi FUJITA[1)]

[1)]Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[2)]Translational Research Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[3)]One Health Research Center, Hokkaido University, Hokkaido, Japan
[4)]Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa
[5)]Biomedical Sciences Department, School of Veterinary Medicine, The University of Zambia, Lusaka, Zambia

ABSTRACT. Wildlife is exposed to a wide range of xenobiotics in the natural environment. In order to appropriately assess xenobiotic-induced toxicity in wildlife, it is necessary to understand metabolic capacities. Carnivores, in general, have low metabolic abilities, making them vulnerable to a variety of chemicals. Raccoons (*Procyon lotor*) in the wild have been found to have high levels of xenobiotics. However, little is known about the metabolic capacity of the cytochrome P450 (CYP) enzymes in this species. Thus, this study used liver samples to investigate the characteristics of CYP enzymes in wild raccoons. In 22 wild raccoons, CYP concentrations in hepatic microsomes were examined. To better understand the properties of CYP-dependent metabolism, *in vitro* metabolic activity studies were performed using ethoxyresorufin, pentoxyresorufin and testosterone as substrates. In addition, three raccoons were fed commercial dog food in the laboratory for one week, and the effects on CYP-dependent metabolism were investigated. In comparison to other mammalian species, raccoons had very low concentrations of CYP in their livers. In an *in vitro* enzymatic analysis, raccoons' ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-depentylase (PROD) metabolic capacities were less than one-fifth and one-tenth of rats', respectively. These results indicate the possible high risk in raccoons if exposed to high levels of environmental xenobiotics because of their poor CYP activity. In this study, the features of CYP-dependent metabolism in wild raccoons are described for the first time.

KEYWORDS: cytochrome P450, raccoon, testosterone, xenobiotics, xenobiotic metabolism

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Wild mammals are exposed to a variety of environmental xenobiotics such as pesticides, organic pollutants including dioxins, and heavy metals, and toxic effects have been reported all over the world [3, 4, 39]. For instance, pesticides, such as rodenticides and insecticides, were detected in more than 1,000 samples of wild mammals between 2005 and 2014 in Italy, and some of the wild mammals were regarded to have died due to exposure to these chemicals [5]. In addition, dioxins and polychlorinated biphenyls (PCBs) were detected at high frequencies in deer and wild boars in Europe despite recent reductions in the amounts of these chemicals used, and the detected doses were thought to be above the thresholds for toxicological effects for these wild mammal species [60]. Moreover, the wildlife embryos and fetuses can be exposed to multiple xenobiotics simultaneously, which can negatively affect a wide range of biological mechanisms, including birthrates [20]. For the conservation of wild mammals, it is necessary to predict toxicological effects on non-target species. Metabolic ability is generally an extremely important factor in estimating chemical sensitivity. Chemicals taken into the body are metabolized mainly in the liver by xenobiotic-metabolizing enzymes (XMEs). In mammals, Cytochrome P450 (CYP) is one of the most significant XMEs involved in chemical elimination [28]. A vast range of substances, including xenobiotics and endogenous compounds, are catalyzed by CYP [19, 28, 32, 55, 56]. Environmental contaminants, such as polychlorinated dibenzo-p-dioxin (PCDDs), polychlorinated dibenzofuran (PCDFs), PCBs, polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT), and many other pesticides, are metabolized by CYP [1, 24, 25, 34]. In wild

*Correspondence to: Ishizuka M: ishizum@vetmed.hokudai.ac.jp, Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan
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mammalian species inhabiting polluted environments, CYP should play an important role in the detoxification of these xenobiotics and in maintaining homeostasis.

Previous research characterized the xenobiotic-metabolizing abilities of Canoidea superfamily animals like foxes, bears, dogs, and minks, and found that these animals lacked the ability to catalyze the 7-hydroxylation of propranolol, which is a major catalytic pathway of propranolol in rat hepatic microsomes. Furthermore, not only did minks have low microsomal concentrations of CYP, but they also had low xenobiotic-metabolizing activity [27]. These results suggest that animals of the Canoidea have lower CYP-dependent xenobiotic-metabolizing activities in comparison with experimental animals, such as rats and mice. Furthermore, several Canoidea animals are apex predators in their ecosystems, and biomagnification causes them to accumulate large amounts of pollutants [51, 58]. Due to their low CYP-dependent metabolic abilities and high levels of pollutant exposure, Canoideas may be high-risk species for xenobiotics. Therefore, determining the characteristics of CYP-dependent metabolic abilities in Canoidea species is critical for estimating their chemosensitivity.

Raccoons (*Procyon lotor*) were imported into Japan from North America in the 1960s and are now distributed in most areas as an invasive alien species [21, 23]. The raccoon is a member of the Canoidea, and these animals now inhabit suburban agricultural areas and eat crops in Hokkaido, Japan [47]. Raccoons could be exposed to chemicals, such as pesticides, by eating contaminated crops. Indeed, the levels of PBDEs found in wild raccoons in the USA were higher than in other terrestrial mammals [6]. However, little information is available regarding CYP-dependent metabolism in raccoons. In this study, we characterized the CYP-dependent metabolism of exogenous and endogenous substrates in raccoons.

MATERIALS AND METHODS

Chemicals

Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Oriental Yeast Co. (Tokyo, Japan). Glucose-6-phosphate (G6P) and G6P-dehydrogenase (G6PDH) were obtained from Sigma (St. Louis, MO, USA). Testosterone, ethoxyresorufin, pentoxyresorufin, and anti-rat CYP antibodies were obtained from Daiichi Chemical Co. (Osaka, Japan). Diaminobenzidine was obtained from Kanto Chemical Co. (Tokyo, Japan).

Animals and treatments

All animal tests were carried out per Hokkaido University's Institutional Animal Care and Use Committee requirements. The raccoons were caught as part of an invasive alien species extermination program in suburban areas in Hokkaido, northern Japan, between April and October 1999. In the present study, 10 males and 12 females were analyzed. Animals were euthanized by CO_2 inhalation, and then the livers of raccoons were collected within 3 hr of their deaths. The removed livers were immediately frozen in liquid nitrogen and stored at −80°C until the assay after removing blood by KCl flushing through the portal vein. In addition, two males and one female were fed commercial dog food (dry food: Vita-One, Nippon Pet Food Co., Ltd., Tokyo, Japan) in the laboratory for 1 week, after which they were sacrificed by the same method as described above. Individual data of captured raccoons, including sex, estimated age, body weight, and month of capture, are shown in Supplementary Table 1. Based on a previous study of raccoon growth, the body weight of raccoons was 3 kg at 6 months old and increased with age until 20 months old [16]. Therefore, age was estimated by body weight: >3 kg for an adult and <3 kg for a cub.

Microsomal fraction preparation

Omura and Sato's method [46] was used to obtain hepatic microsomal samples. Hepatic tissue samples of about 5 g were homogenized in 15 ml of KCl buffer and then centrifuged for 20 min at 9,000 *g* at 4°C. The microsomal pellet obtained by centrifugation was resuspended in 5 mL of KCl solution after the obtained postmitochondrial supernatants (S9 fraction) were centrifuged at 105,000 *g* for 60 min. Samples were centrifuged again for 60 min at 105,000 *g*. Following that, the cleaned microsomal fraction was suspended in a 0.1 M potassium phosphate buffer (pH 7.4). Microsomes were stored at −80°C until analyses. The Lowry method was used to measure the protein concentrations in hepatic microsomes [43]. The total CYP content was estimated using Omura and Sato's technique [46] by examining the reduced CO spectra between 400 and 500 nm with a spectrofluorometer (FP-777; JASCO, Tokyo, Japan).

CYP-dependent activities

To evaluate the CYP-dependent metabolism in raccoons, *in vitro* metabolic assays were performed using ethoxyresorufin, pentoxyresorufin, and testosterone as substrates. Ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-dependentylase (PROD) activities are generally used for the evaluation of CYP1A and CYP2B dependent metabolism, respectively in rats [12, 50]. Testosterone is a typical substrate for several CYP isoforms including CYP2C11, CYP3A, CYP2A, and CYP2B in rats [49, 63].

EROD and PROD activities were measured fluorometrically as previously described [7]. The fluorescence of resorufin was measured using a spectrofluorometer with excitation and emission wavelengths of 528 nm and 590 nm, respectively [9]. The testosterone metabolites were determined using a SUPELCOSIL LC-318 column (SPELCO, Bellefonte, PA, USA) and liquid chromatography (LC-6A; Shimadzu, Kyoto, Japan) according to Hoeven and Sanwald's method [22, 53]. The mobile phase flow rate was 1.0 mL/min. In a 1 mL total incubation mixture, an NADPH-generating system (0.5 mM NADPH, 10 mM G6P, 2 units of G6PDH, and 10 mM magnesium chloride), potassium phosphate buffer (100 mM, pH 7.4), hepatic microsomal (1 mg protein) and substrate (0.25 mM testosterone, 20 µM pentoxyresorufin, and 20 µM ethoxyresorufin) were included. The enzymatic reactions were started by adding NADPH and G6PDH and incubated at 37°C for 10 min. All assays were carried out under conditions that allowed for linear time and

protein concentration reactions. Concentration of each substrate was enough to reach the plateau in the Michaelis-Menten plot and obtained velocity value was regarded as Vmax.

Western analysis of CYP1A1 and CYP2B1 proteins

We performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 7.5% and 10% polyacrylamide gels for CYP1A1 and CYP2B1, respectively, according to the Laemmli method [40]. Authentic standards of CYP isoforms were used in western blotting analyses. Specific antibodies were used to identify the CYP isoforms that were deposited onto nitrocellulose filters, and they were immunostained with diaminobenzidine as a substrate. Antibodies against rat CYP1A1 and 2B1 which were were purchased from Daiichi Pure Chemicals (Tokyo, Japan). After scanning the membrane, NIH Image software 1.61 (National Institutes of Health, Bethesda, MD, USA) was used to evaluate digital images of the immunoblot spectrum configurations [41].

Statistical analysis

Statistical analyses were performed using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). Wilcoxon's test was used to compare CYP concentrations and PROD activities according to sex and age. Welch's *t*-test and Student's *t*-test were used for statistical analysis of EROD activities and testosterone metabolic abilities according to sex and age, respectively. $P<0.05$ was regarded as statistical significance.

RESULTS

CYP content in the liver

CYP concentrations in the hepatic microsomes are shown in Table 1. There was no difference in CYP concentration between males (0.035 ± 0.01 nmol/mg protein) and females (0.071 ± 0.03 nmol/mg protein). In contrast to rats (*Rattus norvegicus*), minks (*Mustela vison*), bears (*Ursus arctos*), foxes (*Vulpes vulpes*), and dogs (*Canis lupus familiaris*) with CYP levels of 0.65, 0.31, 0.75, 0.93, and 0.67 nmol/mg protein, respectively, raccoons' CYP levels were lower than those of other mammals [27]. In addition, three raccoons maintained in the laboratory on commercial dog food for one week showed approximately five-fold higher CYP content than untreated raccoons (0.371 ± 0.08 pmol/min/mg vs. 0.068 ± 0.012 pmol/min/mg, respectively). There was no significant difference between adults and cubs.

EROD activity

Raccoons showed EROD metabolic ability, which is catalyzed mainly by CYP1A1 in rats, mice, and humans [12]. We detected EROD activity in all samples in this study with relatively large SD value (males, 18.1 ± 17.9 pmol/min/mg; females, 20.6 ± 16.5 pmol/min/mg) (Table 2). There were no significant differences in EROD activity according to sex or age. The EROD metabolic activities in raccoons were less than one-fifth of those in rat hepatic microsomes and less than one-fiftieth of those of horses described in our previous study [11].

Raccoons kept in the laboratory for 1 week showed higher activity of EROD compared to untreated raccoons (78.6 ± 16.8 pmol/min/mg vs. 21.8 ± 17.1 pmol/min/mg, respectively) (Table 2).

In western blotting analyses using an anti-CYP1A1 antibody, higher EROD activity was associated with more CYP isoforms responding to this antibody. We showed a positive correlation between CYP content and EROD activity (R^2=0.769) (Fig. 1). These observations indicate that the metabolism of EROD is dependent on this CYP1A isoform in raccoons.

Table 1. The cytochrome P450 amounts of raccoons in this study and other mammals

Animals		Cytochrome P450 concentrations (nmol/mg microsomal protein)
Raccoons	No treated Male (n=8)	0.035 ± 0.01
	No treated Female (n=11)	0.071 ± 0.03
	One week-bred (n=3)	0.371 ± 0.08
Rats	Control (n=3, male)	0.65 ± 0.07
Minks	Control (n=3, male)	0.31 ± 0.05
Bears	Control (n=3, male)	0.75 ± 0.04
Foxes	Control (n=3, male)	0.93 ± 0.07
Dogs	Control (n=3, male)	0.67 ± 0.08

Hepatic microsomal CYP concentrations were determined from carbon monoxide-difference of the reduced microsomes in the livers of raccoons in this study. The results of CYP concentrations of other mammals in the previous studies are also shown, representing the mean ± SD.

Table 2. Hepatic metabolic abilities of ethoxyresorufin O-deethylase (EROD) in raccoons, rats, deer, cattle, and horses

Animals		EROD activity (pmol/min/mg protein)
Raccoons	No treated Male (n=8)	18.1 ± 17.9
	No treated Female (n=11)	20.6 ± 16.5
	One week-bred (n=3)	78.6 ± 16.8
Rats	Control (n=3, female)	107.5 ± 10.5
Deer	Control (n=3, female)	241.2 ± 8.10
Cattle	Control (n=3, female)	337.1 ± 2.40
Horses	Control (n=3, female)	918.2 ± 53.4

Hepatic metabolic activities of EROD were determined fluorometrically in this study. The results of other mammals in the previous study are also shown. Each value represents the mean ± SD.

PROD activity

We demonstrated that raccoon hepatic microsomes possessed PROD metabolic activity, which is catalyzed mainly by the CYP2B subfamily in rats (males, 2.70 ± 1.22 pmol/min/mg; females, 3.12 ± 1.43 pmol/min/mg) (Table 3). There were no differences in the levels of PROD activity according to sex or age, but we found higher PROD activity in the raccoons kept in the laboratory for one week in comparison to the untreated raccoons (9.28 ± 3.42 pmol/min/mg vs. 3.04 ± 1.32 pmol/min/mg) (Table 3).

In western blotting analyses using the anti-CYP2B1 antibody, we observed a correlation between CYP content and PROD activity in female raccoons (R^2=0.811) (Fig. 2) although there was no such correlation in males (R^2=0.087) (Fig. 3).

The amount of CYP that responded to anti-rat CYP2B1 antibody was lower than that of anti-rat CYP1A1 antibody (CYP2B1 antibody, approximately 1–7 pmol/mg protein; CYP1A1 antibody, approximately 10–70 pmol/mg protein) (Figs. 1–3).

Testosterone metabolism

Testosterone metabolites were determined by *in vitro* metabolic assay using hepatic microsomes. In this study, 7α-, 16α-, 2β-, and 6β-hydroxylation were detected, and 6β- and 16α-hydroxytestosterone were abundant. Neither 2α- nor 16β-hydroxylation was detected in any of the raccoons used in this study (Fig. 4). As observed previously in rats [2], male raccoons had significantly higher 6β-hydroxylation activities than female raccoons (P<0.05). There was no significant difference between adults and cubs in testosterone hydroxylation. A correlation was observed between the levels of 6β- and 2β-hydroxylation (R^2=0.980) (Fig. 5).

DISCUSSION

CYP-dependent xenobiotic metabolism in raccoons

There is no molecular information about CYP isoforms of raccoons. Although no genetic analysis of raccoon CYP isoforms was performed in this study, some characteristics of CYP1, 2, and 3 isoforms were estimated by the results of EROD, PROD, and testosterone metabolism as described below.

It has been reported that CYP1A subfamilies are present in most vertebrate animals, and they show high degrees of identity between species [17]. This study showed that raccoons also have the CYP1A isoform, and CYP1A-dependent activities were observed in EROD metabolism as in other animals. This was consistent with previous studies indicating that CYP1A is homologous between animal species; CYP1A can be considered as the most conserved isoform within the phylogenetic tree so that CYP1A proteins share extensive structural similarity and display similar specificity to the orthologous among different animal species [26]. CYP1A-dependent metabolic abilities were found in all raccoons in this study. However, the metabolic activities of CYP in raccoons were much lower than those of rats and other animals. The EROD metabolic activities in raccoons were less than one-fifth of those in rat hepatic microsomes and less than one-fiftieth of those of horses described in our previous study [11]. CYP1A subfamilies play important roles in the detoxification of environmental pollutants, such as aromatic hydrocarbons, carbamate insecticides, several organophosphorus, and rodenticide i.e., warfarin [13, 29, 33, 57, 61]. Therefore, raccoons may have increased susceptibility to environmental pollutants

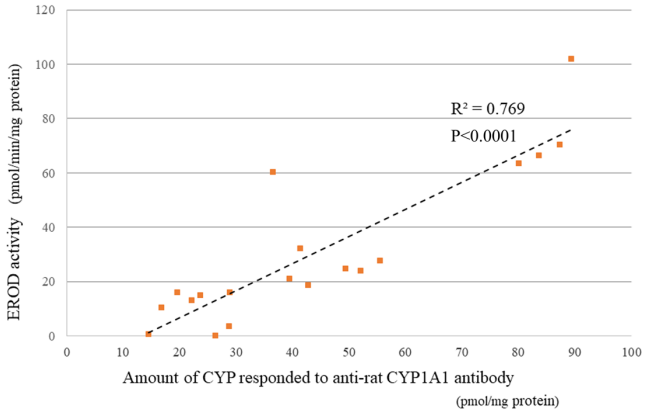

Fig. 1. Correlation between the ethoxyresorufin O-deethylase (EROD) activity and the amounts of cytochrome P450 (CYP) which responded to anti rat CYP1A1 antibody. Vertical axes show the EROD metabolic ability. EROD metabolic activities in the liver of raccoons were measured fluorometrically by *in vitro* assay. Horizontal axes represent the amounts of CYP isoforms which responded to anti-rat CYP 1A1 antibody. Responded CYP isoforms of raccoons were identified by western blotting using anti-rat CYP1A1 antibody. Dashed line shows the positive correlation between the EROD activity and the amount of responded CYP in raccoons (R^2=0.769).

Table 3. Hepatic metabolic abilities of pentoxyresorufin O-depentylase (PROD) in raccoons and rats

Animals		PROD activity (pmol/min/mg protein)
Raccoons	No treated Male (n=8)	2.70 ± 1.22
	No treated Female (n=11)	3.12 ± 1.43
	One week-bred (n=3)	9.28 ± 3.42
Rats	Control (n=3, female)	48.5 ± 8.08

Hepatic metabolic activities of PROD were determined fluorometrically in this study. The results of rats in previous study are also shown, representing the mean ± SD.

that are metabolized mainly by CYP1A isoforms. Additionally, raccoons showed large SD values of EROD activity compared to that of PROD activity. CYP1A isoforms are relatively easily induced by various chemicals such as PCBs and benzo[α]pyrene [12, 17], and individual differences in exposure levels of environmental pollutants in raccoons might have increased the variability in CYP1A activity.

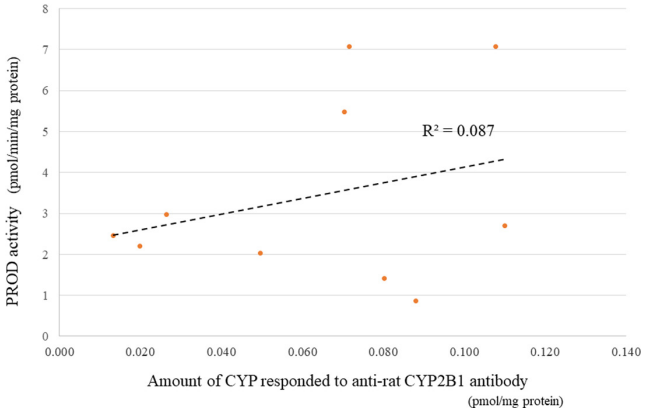

Fig. 2. Correlation between the pentoxyresorufin O-dependentase (PROD) activity and the amounts of cytochrome P450 (CYP) which responded to anti rat CYP 2B1 antibody in male raccoons. Vertical axes show the PROD metabolic ability. PROD metabolic activities in the liver of raccoons were measured fluorometrically by *in vitro* assay. Horizontal axes represent the amounts of CYP isoforms which responded to anti-rat CYP 2B1 antibody. Responded CYP isoforms of raccoons were identified by western blotting using anti-rat CYP2B1 antibody. Dashed line shows no significant correlation between the PROD activity and the amount of responded CYP in male raccoons (R^2=0.087).

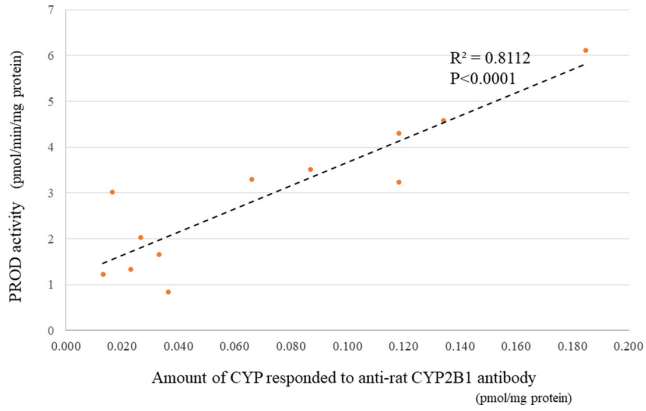

Fig. 3. Correlation between the pentoxyresorufin O-dependentase (PROD) activity and the amounts of cytochrome P450 (CYP) which responded to anti rat CYP 2B1 antibody in female raccoons. Vertical axes show the PROD metabolic ability. PROD metabolic activities in the liver of raccoons were measured fluorometrically by *in vitro* assay. Horizontal axes represent the amounts of CYP isoforms which responded to anti-rat CYP 2B1 antibody. Responded CYP isoforms of raccoons were identified by western blotting using anti-rat CYP2B1 antibody. Dashed line shows the positive correlation between the PROD activity and the amount of responded CYP in female raccoons (R^2=0.811).

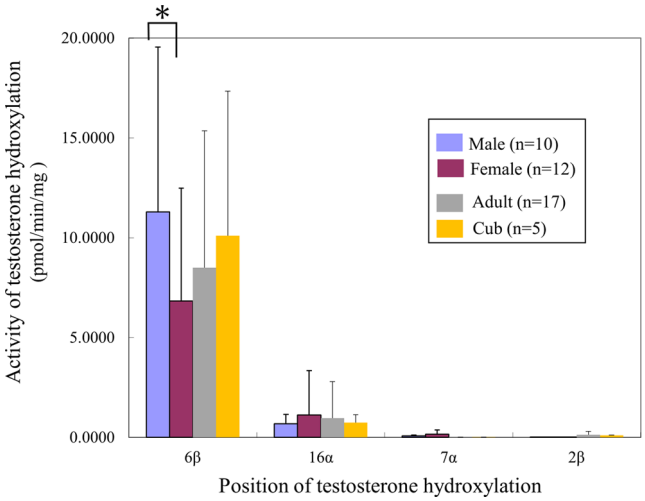

Fig. 4. Activities of testosterone hydroxylation formed in hepatic microsomes of raccoons (n=10 for males, n=12 for females). Metabolic abilities of testosterone hydroxylation in raccoons were measured by *in vitro* assay using hepatic microsomes. Vertical axes indicate the activity of testosterone hydroxylation. Each data point for the mean of activity and error bar represents the S.D value. In this study, 7α-, 16α-, 2β-, and 6β-hydroxylation were detected, and 6β- and 16α-hydroxy testosterone were abundant. In the 6β hydroxy testosterone, significant difference was observed between males and females (P<0.05).

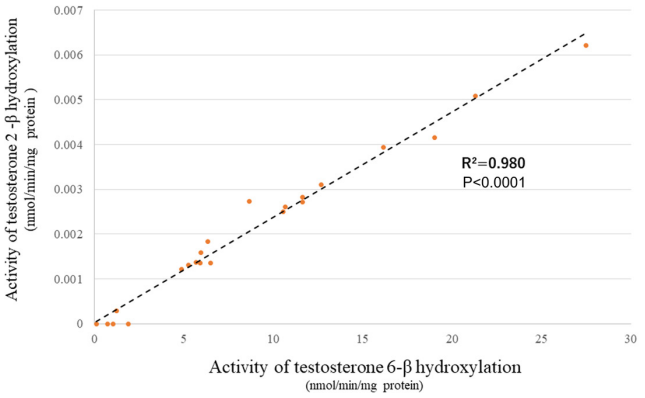

Fig. 5. Correlation between the levels of 6β- and 2β- hydroxylation in hepatic microsomes of raccoons. Metabolic abilities of testosterone hydroxylation in raccoons were measured by *in vitro* assay using hepatic microsomes. Vertical axes and horizontal axes indicate the metabolic abilities of testosterone 2β- and 6β-hydroxylation, respectively. Dashed line shows strong positive correlation between the activity of testosterone 2β- hydroxylation and 6β- hydroxylation (R^2=0.980).

The levels of PROD activity in the livers of dogs were shown to be almost the same as those of rodents [53]. However, in this study, the PROD activity in raccoons was lower than in rats. The PROD activities of raccoons were less than one-tenth of those of rats in the previous study [10]. CYP2B isoforms have low substrate specificity but are involved in the detoxification of a wide range of chemicals, including organochlorine insecticides [42]. DDT and DDE were found in all of the raccoons examined in a previous study [45]. Considering the low metabolic abilities in the liver and high exposure rates, these environmental pollutants may have some negative toxicological effects on wild raccoons. Also, a positive correlation between CYP concentration and PROD activity in the liver was observed in females but not in males. Therefore, it seems that, unlike rats, PROD is catalyzed by CYP isoforms that are differentially expressed between males and females in raccoons [48]. It is known that CYP1A2 and CYP2C13 are involved in PROD activity in addition to CYP2B1 in rat [37], and PROD activity might be catalyzed by CYP isoforms other than CYP2B in male raccoons. Furthermore, on western immunoblotting analysis, fewer raccoon CYP proteins cross-reacted with rat anti-CYP2B antibody in comparison to the results with rat anti-CYP1A antibody. This difference between CYP1A and CYP2B in the amount of protein cross-reactive with rat anti-CYP antibody was also observed in a previous study using hepatic microsomal CYP of seal species [8]. This could be because CYP2B subfamilies have evolved independently and diversified among animal species, unlike CYP1A subfamilies [35, 36].

Testosterone metabolism by CYP in raccoons

Testosterone is metabolized by several CYP isoforms, such as CYP2C11, CYP3A, CYP2A, and CYP2B subfamilies, in male rats [49, 63]. We detected 6β-, 16α-, 7α-, and 2β-hydroxytestosterone as metabolites of testosterone in raccoon hepatic microsomes, and 6β- and 16α-hydroxy metabolites were abundant. These observations were consistent with previous research showing that canine hepatic microsomes hydroxylated testosterone mainly at the 6β and 16α positions [30]. In raccoons, we observed strong correlations between testosterone metabolic activities at 6β and 2β positions. In rat hepatic microsomes, CYP3A subfamilies catalyzed testosterone 6β- and 2β-hydroxylation [59, 63]. Moreover, both CYP3A12 and CYP3A26 in canines catalyze testosterone 6β- and 2β-hydroxylation [14]. We, therefore, suggest that the same isoforms of CYP3A could catalyze both 6β- and 2β-hydroxylation in raccoon hepatic microsomes as they do in rat and canine liver microsomes.

The 16α position of testosterone is hydroxylated mainly by CYP2C11 in rat hepatic microsomes [63]. CYP2C11 and CYP3A subfamilies are regulated by several hormones, and their expression levels change with age in rats [31]. However, there were no significant differences in testosterone metabolic abilities between adult and juvenile raccoons in this study. Therefore, CYP isoforms that are not dependent on age appear to mainly catalyze testosterone in raccoons, unlike rats.

Monitoring of environmental pollution using CYP as a biomarker in wildlife

CYP-dependent activities of wildlife have been used for the monitoring of environmental pollution levels because the expression levels of CYP isoforms are induced by xenobiotics [52]. CYP1A subfamilies in particular are used as biomarkers for dioxins and PAHs exposure [38, 62]. Non-planar PCBs, DDT, and other chlorinated pesticides induce CYP2 and CYP3A subfamilies in wildlife [15]. Overall, these CYP isoforms seem to be useful as biomarkers to determine the levels of environmental pollutants. Indeed, CYP-dependent activities, especially CYP1A1 and CYP1A2 activities, are reported to be related to PCB residue levels in wild raccoons [54]. In this study, raccoons fed commercial dog food in the laboratory for one week showed elevations in CYP concentration, EROD activity, and PROD activity. It was reported that chemical contaminants, such as PCBs, which were detected in several dog food products [44], and there was possibility to induced CYP in these raccoons. However, nutrition changes in dog food, especially protein and lipids, might have mainly affected the metabolic activities of some CYP isoforms [18]. In any case, larger interindividual differences in metabolic abilities were observed in raccoons than in experimental animals, and it is necessary to consider confounding factors when analyzing the xenobiotic-metabolizing activities of wild animal species.

Conclusion

The present study indicates that raccoons have lower CYP concentrations in their livers, as well as lower CYP-dependent metabolic activities compared to other mammalian species. This research will be helpful in the understanding of xenobiotic metabolism and the prediction of the hazardous effects of environmental toxins on wild raccoons, leading to better conservation of other mammalian carnivore species.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

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