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The African hedgehog (*Atelerix albiventris*): Low phase I and phase II metabolism activities

Aksorn Saengtienchai [a,b], Yoshinori Ikenaka [a,c,*], Nesta Bortey-Sam [a], Usuma Jermnark [b], Hazuki Mizukawa [d], Yusuke K. Kawai [e], Shouta M.M. Nakayama [a], Mayumi Ishizuka [a]

[a] Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9, Kita–ku, Sapporo 060–0818, Japan
[b] Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, 50 Ngam Wong Wan Rd, Latyaow, Chatuchak, Bangkok 10900, Thailand
[c] Water Research Group, Unit for Environmental Sciences and Management, North–West University, Potchefstroom, South Africa
[d] Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9, Kita–ku, Sapporo 060–0818, Japan
[e] Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, ,Obihiro 080–8555, Japan

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ABSTRACT

The African hedgehog, *Atelerix albiventris*, is a spiny mammal that has become popular as an exotic pet in many countries. To elucidate the ability of hedgehogs to metabolize xenobiotics, the animals were exposed to polycyclic aromatic hydrocarbon, pyrene. The *in vivo* exposure study indicated that pyrene was biotransformed to glucuronide and sulfate conjugates, such as pyrene-1-glucuronide, pyrene-1-sulfate, and pyrenediol-sulfate, and excreted in the urine. Pyrene-1-glucuronide was the main metabolite, and limited sulfate conjugate excretion was observed. The main products excreted in feces were 1-hydroxypyrene and pyrene. Based on the results of the *in vivo* exposure study, *in vitro* enzymatic kinetic experiments were performed using various substrates and compared to rats and pigs. The enzyme efficiencies of cytochrome P450 (CYP)-mediated ethoxyresorufin *O*-deethylase activity and warfarin 4′-, 6-, and 8-hydroxylation activity in hedgehogs were lower than those of rats. Furthermore, UDP-glucuronosyltransferase activity in hedgehogs also had a lower K_m value than that in pigs. Interestingly, the enzyme efficiencies of sulfation activity toward 1-hydroxypyrene and β-estradiol in hedgehogs were significantly lower than those in pigs. These observations suggested that phenol and estrogen sulfotransferases may have limited roles in xenobiotic metabolism in hedgehogs.

1. Introduction

The African hedgehog, *Atelerix albiventris*, is a spiny-coated insectivore that is popular as an exotic pet worldwide. Wild hedgehogs normally eat insects, slugs, small mammals, frogs, worms, and some fruits, and live in urban and suburban areas (Ivey and Carpenter, 2012). Hedgehogs are generally fed commercial cat food (Heatley, 2009; Ivey and Carpenter, 2012). The diet and lifestyle of hedgehogs living close to humans suggest that they may be exposed to various chemicals and pollutants (D'Have et al., 2007; Vermeulen et al., 2010). Recently, hedgehogs were suggested to be non-target animals for anticoagulant rodenticides. Due to their lifestyle, hedgehogs could be exposed to anticoagulants both directly and indirectly (Dowding et al., 2010). Moreover, hedgehog hair is a biomarker for heavy metal bioaccumulation. Heavy metal levels in hedgehogs are related to the levels in the biota, including the soil and worms (D'Have et al., 2007). In addition, other persistent compounds, such as organochlorine and organobromine, were also shown to accumulate in hedgehogs (Vermeulen et al., 2010). However, little is known about the xenobiotic metabolism system, which strongly affects chemical sensitivity, of hedgehogs.

The most important xenobiotic metabolizing enzyme family in phase I reaction is cytochrome P450 (CYP) (Martignoni et al., 2006). The phase II conjugation enzymes, such as UDP- glucuronosyltransferase (UGT), sulfotransferase (SULT), and glutathione-S-transferase, *etc*., change the physicochemical characteristics of phase I metabolites to more water-soluble forms (or conjugated forms) before excretion into the urine and feces. Interspecies differences in phase I oxidation and phase II conjugation reaction have been reported (Court, 2001; Darwish et al., 2010; Saengtienchai et al., 2014; Watkins and Klaassen, 1986). The total contents of hepatic CYP are different among rats, pigs, rabbits, dogs, and cats (Watkins and Klaassen, 1986). Furthermore, the metabolic activities toward ethoxyresorufin *O*-deethylation, benzo(a)pyrene hydroxylase activity, benzphetamine *N*-demethylase activity, and ethylmorphine *N*-demethylase activity show differences between various mammal species (Darwish et al., 2010; Watkins and Klaassen, 1986). In phase II conjugation, isoforms of UGT1A6, 1A7, and 1A9 are mainly related to glucuronidation of various xenobiotic compounds (Luukkanen et al., 2001). It had been reported that UGT1A6 is dysfunctional or a pseudogene in the Felidae family, including cats

* Corresponding author at: Water Research Group, Unit for Environmental Sciences and Management, North–West University, Potchefstroom, South Africa.
E-mail address: y_ikenaka@vetmed.hokudai.ac.jp (Y. Ikenaka).

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(Court, 2013). Recently, UGT1A6 isoforms were also classified as pseudogenes in brown hyenas, northern elephant seals, northern fur seals, and Steller sea lions (Kakehi et al., 2015; Shrestha et al., 2011). Similar to cats, these mammals may have weak glucuronidation activity toward xenobiotics.

Sulfation is also known to be a major phase II conjugation reaction for the detoxification of numerous xenobiotic compounds in humans and other mammals. Cytosolic sulfotransferases (SULTs) are the enzymes that mediate transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to acceptor substrate compounds containing a hydroxyl or amino group (Coughtrie, 2002). Sulfate conjugation by the SULT enzymes leads to inactivation of biologically active compounds and/or increases in their water solubility, thereby facilitating their removal from the body. Mammalian SULTs have been classified into SULT1, SULT2, SULT3, SULT4, and SULT5 families (Gamage et al., 2006). The various SULT isoforms exhibit metabolic activities toward endogenous (SULT1A, SULT2A, and SULT1E) and xenobiotic (SULT1A, SULT1B, and SULT1C) compounds (Coughtrie, 2002; Harris et al., 2004). In addition, isoforms of human SULTs have also been predicted to be pseudogenes (SULT1D2P, SULT3A1P, SULT1C1P, SULT1D1P, and SULT2A1P) (Freimuth et al., 2004). Generally, pigs have poor sulfation ability against xenobiotic compounds. Recently, limited sulfation has not only been reported in pigs but also in other mammals. With regard to melatonin sulfation, the intrinsic clearance rate in pigs was lower than those in monkeys, rats, dogs, and humans, but higher than that in mice (Tian et al., 2015). In our previous study, pyrene metabolites in urine were used to characterize differences in conjugation reactions between various mammal species. Pyrene is the ubiquitous environmental pollutants and it metabolites are typical phenolic xenobiotics model. The urinary pyrene metabolite is useful to characterize species differences in phase II xenobiotic conjugation reaction (Saengtienchai et al., 2014). The results suggested that pyrene glucuronide is the dominant conjugate excreted *via* urine in the majority of mammals, except cats and ferrets. In contrast, hedgehogs and pigs have limited pyrene sulfate (Saengtienchai et al., 2014). Although pigs were reported to have low sulfation activity, insufficient information is available regarding xenobiotic metabolism in hedgehogs.

In the present study, the phase I oxidation and phase II conjugation reactions in hedgehogs were investigated. Pyrene was chosen as a model substrate for *in vivo* exposure study. For further confirmation about the enzymatic efficiency of each metabolizing enzymes, ethoxyresorufin *O*-deethylase (EROD) activity (indicator for CYP1A1 activity), pentoxyresorufin *O*-dealkylase (PROD) activity (indicator for CYP2B1 activity), and warfarin 4′-, 6-, 7-, 8-, and 10-hydroxylation (4′-, 6-, 7-, 8-, and 10-OH) activities, which are metabolized by various CYP isoforms, were measured. These CYP enzymes are also contributed to pyrene and warfarin metabolism. To assess the efficiency of phase II conjugation enzymes, 1-hydroxypyrene was chosen to measure UGT-dependent activity, and 1-hydroxypyrene and β-estradiol were used to estimate SULT-dependent activities.

2. Material and methods

2.1. Animals

Ten-month-old male African hedgehogs (*Atelerix albiventris*) ($n = 3$) were obtained from a local pet shop (Bangkok, Thailand) and acclimated for 2 weeks in the laboratory. The hedgehogs had an average ± SD body weight of 298 ± 46 g. The animals were given commercial cat food (Me-O, Perfect Companion group, Thailand) and clean water *ad libitum*. Eight-week-old, male Wistar rats (*Rattus norvegicus*) ($n = 3$) were obtained from National Laboratory Animal Center, Mahidol University (Bangkok, Thailand) and acclimated for 1 week in the laboratory. The body weights of rats (mean ± standard deviation (SD)) were 365 ± 10 g. The animals were kept under conditions of 40% humidity at 25 °C in a temperature-controlled room with a 12-h light/dark cycle. The animals were given laboratory food (National Laboratory Animal Center, Thailand) and clean water *ad libitum*. All animal experiments were performed under the supervision and with the approval of the Institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, Kasetsart University. Pig liver samples were collected from five male large white pigs (*Sus scrofa domesticus*) at the age of 5 months from a slaughterhouse in Hokkaido, Japan. Samples were immediately flash-frozen in liquid nitrogen and kept at −80 °C until analysis.

2.2. Chemicals

Methanol, acetonitrile, acetic acid, and ammonium acetate were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Pyrene, 1-hydroxypyrene, 3′-phosphoadenosine 5′-phosphoslfate (PAPS), resorufin, resorufin ethyl ether, and 7-hydroxy-3H-phenoxazine-3-one were obtained from Sigma-Aldrich Co. (St. Louis, MO). 6-Hydroxychrysene (6-OH chrysene) as an internal standard was purchased from AccuStandard Inc. (New Haven, CT). UDP-glucuronic acid (UDP-GA) and warfarin sodium were purchased from Wako (Osaka, Japan). D-Glucose-6-phosphate-disodium salt, β-nicotinamide-adenine dinucleotide phosphate, and glucose-6-phosphate dehydrogenase were from Oriental Yeast Co., Ltd. (Tokyo, Japan). Warfarin metabolites (4′-, 6-, 7-, 8-, and 10-hydroxywarfarin) were obtained from Ultrafine Chemicals (Manchester, UK). Pyrene-1-glucuronide, pyrene-1-sulfate, and pyrenediol sulfate were obtained from TOPU Bio (Toyama, Japan). All chemicals used for high-performance liquid chromatography (HPLC) and mass spectrometry (MS) were of HPLC or MS grade, respectively, and were obtained from Kanto Chemical Co. Inc.

2.3. Pyrene exposure and sample collection

Hedgehogs and rats were fasted for 24 h before exposure to the test chemicals. Pyrene was dissolved in 100% propylene glycol and administered orally at a dose of 4 mg/kg body weight. Animals were then kept in a metabolic cage for 24 h for urine and feces collection. After 24 h, urine (hedgehog urine volume: 2.2 ± 1.4 mL; rat urine volume: 10.0 ± 2.2 mL) and feces (1.0 ± 0.3 g, 1.6 ± 1.0 g for hedgehogs and rats, respectively) samples were collected and kept at −20 °C until analysis. The animals were then anesthetized with isoflurane and sacrificed with CO_2. The livers were removed and perfused with cold 1.15% potassium chloride to remove blood, and samples were immediately placed in liquid nitrogen and kept at −80 °C.

2.4. Extraction of pyrene metabolites in urine and feces

Samples were extracted and cleaned up according to Saengtienchai et al. (2015). Briefly, samples of 1 mL of urine were extracted with the same amount of 70% methanol, and 10 μL of 200 ppm 6-OH Chrysene (dissolved in methanol) was added as an internal standard. The mixtures were extracted by vortexing and centrifugation at 9000 ×*g* for 10 min at 4 °C. Then, the supernatants were filtrated with a 0.2 μm syringe filter (SupraPure; Recenttec, Tokyo, Japan). Filtered samples were kept at −20 °C until analysis.

Feces samples of approximately 1 g were homogenized with 20 mL of 70% methanol, and 75 μL of internal standard. The mixtures were then extracted by sonication for 20 min and centrifuged at 9000 ×*g* for 10 min at 4 °C. The supernatants were transferred into 50-mL Falcon tubes. The residual samples were extracted again. The pooled supernatants were filtrated with a 0.2 μm syringe filter and kept at −20 °C until analysis.

Aliquots of 5 μL of filtrated samples were analyzed by high-performance liquid chromatography with fluorescence detection (HPLC/FD) and liquid chromatography mass spectroscopy (LC-MS/MS). The pyrene conjugated metabolites as pyrene-1-glucuronide, pyrene-1-sulfate, and pyrenediol-sulfate (M2 and M3 compounds) were