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Inter-species comparison of liver and small intestinal microsomal metabolism of fluoranthene

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Abstract

The magnitude of susceptibility to toxicant exposure may depend on the ability of animals to metabolize the chemicals. The present study has been undertaken to see whether any differences exist among mammals in the metabolism of fluoranthene (FLA), a polycyclic aromatic hydrocarbon (PAH) compound. Microsomes were isolated from the small intestine and liver of rat, mouse, hamster, goat, sheep, pig, dog, cow, monkey, and humans (commercially procured), and incubated with FLA. Post-incubation, samples were extracted with ethyl acetate and analyzed for FLA/metabolites by reverse-phase HPLC with fluorescence detection. The metabolism of FLA in both liver and small intestine was in the order: human > monkey > cow > goat > sheep > dog > pig > hamster > rat > mouse under conditions of the test system used. The rate of metabolism (pmol of metabolite/min/mg protein) was found to be more in liver than in intestine in all the species studied. The FLA metabolites identified were FLA 2,3-diol, trans-2,3-dihydroxy-1,10b-epoxy-1,2,3,10β tetrahydro FLA (2,3D FLA), 3-hydroxy FLA, and 8-hydroxy FLA. The rodent microsomes produced considerably higher proportion of FLA 2,3-diol, and 2,3D FLA than did pig, dog, and humans. On the other hand, microsomes from higher mammals converted a greater proportion of FLA to 3-hydroxy FLA, the detoxification product of FLA. Overall, our results revealed a great variation among species to metabolize FLA.

Keywords: Fluoranthene; Polycyclic aromatic hydrocarbons; Intestinal microsomes; Liver microsomes; Metabolism; HPLC

1. Introduction

One class of environmental chemicals that is a common contaminant of food is polycyclic aromatic hydrocarbons (PAHs). Automobile exhausts, industrial emissions, cigarette smoke, biomass burning, incense burning, municipal incinerators, and hazardous waste sites release considerable amounts of these chemicals into the environment. Besides environment, processing techniques also contribute to contamination of food by PAHs (reviewed in Ramesh et al., 2004). Approximately 97% of the total daily intake of PAH by humans is through diet (Hattermer-Frey and Travis, 1991). When ingested, PAHs become activated in liver and extra hepatic tissues to reactive metabolites that cause toxicity and interfere with target organ function. Studies have documented an association between the substantially higher levels of dietary and environmental PAH exposures with an increased incidence of esophageal (Roth et al., 1998) and colorectal (Sachse et al., 2002) cancers as well as endocrine disruption-related infertility (Archibong

Abbreviations: CYP, cytochrome P450; FLA, fluoranthene; FLA 2,3-diol, fluoranthene 2,3-dihydrodiol; 2,3-DFLA, trans-2,3-dihydroxy-1,1,1-0b-epoxy-1,2,3,10b tetrahydro fluoranthene; HPLC, high-performance liquid chromatography; 3(OH) FLA, 3-hydroxy fluoranthene; 8(OH) FLA, 8-hydroxy fluoranthene.

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et al., 2002; Inyang et al., 2003). Since the metabolic pathways of PAHs are substrate- (Shimada et al., 2002) and tissue-specific (Shimada et al., 2003), conducting species-specific metabolism studies will be helpful in computing chemical-specific adjustment factors for risk assessment purposes (Walton et al., 2001).

Recent years have seen a surge in the advocacy of using in vitro systems as alternatives to whole animals for toxicological testing purposes. This approach not only helps to reduce the number of animals needed for research but also helps to predict the toxicity for a wide range of animals (NIEHS, 1997). Microsomes from several organs have long been used as one of the in vitro research models to study the metabolism of drugs and toxic chemicals. Microsomes are subcellular organelles that harbor drug metabolizing enzymes necessary for detoxification of chemicals (Ekins et al., 2000). Most of the studies on metabolism of PAHs using liver microsomal preparations have been carried out in rodents, camel, monkey and humans (reviewed ATSDR, 1995; IPCS, 1998). To our knowledge, no information is available concerning the microsomal metabolism of PAHs either in liver or in small intestine of meat animals. Given the fact that edible tissues of some of these animals form an important ingredient of human diet, information on the ability of these animals to process toxic chemicals will be useful to assess risks to humans arising from consumption of contaminated food. Furthermore, knowledge of microsomal biotransformation in higher mammals would help in extrapolation of metabolic data from one species to another.

The intestine is the first functional interface between orally ingested PAHs and other organ systems (Cavret et al., 2004), and the liver is the principal site of PAH metabolism (Wall et al., 1991). Consequently, the rationale for the present study was to look into aspects of intestinal and hepatic microsomal metabolism of fluoranthene (FLA) a PAH compound, by different mammalian species. Fluoranthene was chosen for this study because it is a "nonclassifiable carcinogen" (carcinogenicity class D according to EPA., 2001), widely distributed in the environment (Lobscheid et al., 2004), food contaminant (Ramesh et al., 2004), causes immunosuppression (Yamaguchi et al., 1996), lung and liver tumors (Wang and Busby, 1993), and tubular casts in kidneys (Knuckles et al., 2004).

2. Materials and methods

2.1. Test species/tissue samples

Microsomes were isolated from the liver and intestinal tissues (mainly jejunum) of the following animals: rat (*Rattus norvegicus*; male, aged 8 weeks), mouse (*Musculus domesticus*; male, aged 8 weeks), golden hamster (*Mesocricetus auratus*; male, aged 10 weeks), pig (*Sus scrofa*; aged 9 months), beagle dog (*Canis familiaris*; male, aged 12 months), cow (*Bos Taurus*; aged 18 months), sheep (*Ovis aries*; male, aged 12–24 months), goat (*Capra aegagrus nircus*; male, aged 9–16 months), monkey (*Macaca mulatta*; male, aged 8–16 years), and humans (*Homo sapiens*; mixed pool of both sexes, aged 35–60 years). Microsomes from the small intestine of dogs were a gift from Dr. Thomayant Prueksaritanont, Merck Co. Inc., PA.

Cow, sheep, goat and pig liver and small intestinal samples were obtained from Shackle Island Meats, Hendersonville, TN and A&D Meat Processing, Chapel Hill, TN. Monkey liver and intestines were obtained from the Oregon National Primate Center, University of Oregon. Hamster small intestine and liver samples were provided by Dr. Irma Gimenez-Conti, University of Texas, MD, Anderson Cancer Center and Dr. Gary Olson, Vanderbilt University Department of Cell Biology.

Human intestinal and liver microsomes were purchased from Invitro Technologies Inc. (Baltimore, Maryland). All animals used in this study were mature and healthy and were not subjected to any form of treatment/medication.

2.2. Chemicals

The test chemical FLA (CAS No. 206-44-0; 98% pure) was purchased from the Sigma Chemical Co. (St. Louis, MO). Before use, its purity was confirmed by gas chromatography–mass spectrometry. Fluoranthene metabolite standards were obtained from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO). Methanol, chloroform, and ethanol were purchased from Fisher Scientific Company (Kennesaw, GA). Sucrose, EDTA, and tris–HCl were purchased from Curtin Matheson Scientific Inc. (Houston, TX). Because FLA and its metabolites are suspected carcinogens, they were handled in accordance with NIH guidelines for preventing exposure of lab personnel and equipment to this chemical (NIH, 1981).

2.3. Preparation of microsomes

Immediately following CO_2 asphyxiation (rat, mouse, and hamster), and euthanasia (monkey) liver and small intestinal segments were immediately excised and placed on dry ice prior to storage at −80 °C until isolation of microsomes. Prior appointments were made with the management of abattoirs to procure tissue samples from meat animals (cow, pig, sheep, and goat) within 5 min after the evisceration process. The tissues of interest were immediately frozen and transported to the laboratory on dry ice and subsequently stored frozen at −80 °C until used for microsome isolation.

Intestinal and liver tissues from cow, pig, sheep, and goat were washed in chilled isotonic saline to remove excess blood. The adherent connective tissues and fat were removed. The intestinal lumen was also flushed with isotonic saline to remove partially digested food residues. Liver and intestine were cut into small pieces and pieces from individual organ were thoroughly mixed to obtain a homogenous sample. The samples were weighed, and chilled in isotonic saline. Each tissue was homogenized in two volumes of sucrose-TKM buffer (sucrose 0.25 M, Tris 80 mM, KCl 25 mM, $MgCl_2$ 5 mM, pH 7.4). The homogenate was centrifuged at $10{,}000 \times g$ for 10 min to remove gross tissues and fat. The supernatant was centrifuged at $15{,}000 \times g$ for 15 min to remove nuclei and mitochondria. The post-mitochondrial fraction was centrifuged at $100{,}000 \times g$ for 60 min at 4 °C to pellet down the microsomes. The microsomal pellet was rinsed twice with 5 ml of sucrose-TKM buffer and resuspended in 5 ml of the same buffer. The isolated microsomes were aliquoted into cryovials, frozen in liquid nitrogen until use. Microsomes from dog small intestine were isolated as detailed in Prueksaritanont et al. (1996). Protein content of the microsomal preparations was determined according to the method of Bradford (1976).

2.4. Metabolism studies

Pilot studies were conducted to establish optimal conditions for microsomal protein concentration, substrate concentration and time of incubation of reaction mixtures in the assay. The results (data not shown here)