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Allometric scaling of hepatic biotransformation in rainbow trout

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ABSTRACT

Biotransformation may substantially impact the toxicity and accumulation of xenobiotic chemicals in fish. However, this activity can vary substantially within and among species. In this study, liver S9 fractions from rainbow trout (4–400 g) were used to evaluate relationships between fish body mass and the activities of phase I and phase II metabolic enzymes. An analysis of log-transformed data, expressed per gram of liver (g liver^{-1}), showed that total cytochrome P450 (CYP) concentration, UDP-glucuronosyltransferase (UGT) activity, and glutathione *S*-transferase (GST) activity exhibited small but significant inverse relationships with fish body weight. In contrast, in vitro intrinsic clearance rates ($CL_{IN\ VITRO,INT}$) for three polycyclic aromatic hydrocarbons (PAHs) increased with increasing body weight. Weight normalized liver mass also decreased inversely with body weight, suggesting a need to express hepatic metabolism data per gram of body weight (g BW^{-1}) in order to reflect the metabolic capabilities of the whole animal. When the data were recalculated in this manner, negative allometric relationships for CYP concentration, UGT activity, and GST activity became more pronounced, while $CL_{IN\ VITRO,INT}$ rates for the three PAHs showed no significant differences across fish sizes. Ethoxyresorufin *O*-deethylase (EROD) activity normalized to tissue weight (g liver^{-1}) or body weight (g BW^{-1}) exhibited a non-monotonic pattern with respect to body weight. The results of this study may have important implications for chemical modeling efforts with fish.

1. Introduction

Fish, like mammals, metabolically transform many xenobiotic chemicals into more water-soluble metabolites via phase I and phase II reaction pathways (Schlenk et al., 2008; Sijm and Opperhuizen, 1989). This activity generally results in reduced chemical accumulation and toxicity, although biotransformation may also result in formation of reactive products that are more toxic than the parent compound (i.e., bioactivation). In either case, the potential for a chemical to accumulate in fish and exert a toxic effect may be substantially determined by its rate of biotransformation.

In vitro biotransformation rate and affinity data for fish were previously summarized by Fitzsimmons et al. (2007). These data show that metabolism rates for the same chemical may vary substantially within and among species. Some of these differences can be attributed to species differences in expression of biotransformation enzymes (Wang et al., 2001), while additional variability may be due to methodological differences (e.g., enzyme system; substrate concentration). However, controlled studies indicate that a variety of factors can impact biotransformation rates within the same test animals. For example, Vignier et al. (1996) evaluated the effect of life-stage on hepatic biotransformation by comparing the metabolism of 7-ethoxyresorufin in microsomes collected from Atlantic salmon parr and smolts. Estimates of V_{MAX} were found to be about 10 times greater in smolts. Kennedy and Tierney (2006) investigated the effect of diet and fasting on the metabolism of benzo[*a*]pyrene in rainbow trout. Measured rates of activity in hepatocytes collected from fasted animals were 2 to 3 times lower than those of fed animals. Additional factors shown to influence biotransformation activity include sexual maturity and gender (Stegeman and Chevion, 1980; Koivusaari et al., 1981, 1984; Förlin and Haux, 1990), and acclimation temperature (Karr et al., 1985; Carpenter et al., 1990).

Across the animal kingdom, many metabolic and physiological processes vary with body size (West and Brown, 2005). The relationship between a biological parameter and body mass is commonly described by the allometric equation $Y = a \times M^b$, where Y is the parameter of interest, expressed on a whole animal basis, M is body mass, a is a normalization coefficient, and b is the allometric or scaling exponent. In mammals, many physiological processes scale to body weight with fractional exponents of 0.6 to 0.8 (-0.4 to -0.2 when the data are expressed on a gram of body weight basis [g BW^{-1}]). These relationships are typically developed using data collected from multiple species. In fish, however, it is difficult to combine data for different species

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due to the large impact of water temperature on metabolic and physiological processes. For this reason, allometric relationships for fish are generally developed for a single species. This approach eliminates variability due to interspecies difference in measured processes. In addition, many species exhibit indeterminate growth, resulting in large ranges in body mass.

Basal metabolic rate in fish (measured as oxygen consumption; VO_2) typically scales to body mass with a fractional exponent ranging from 0.6 to 0.9 (-0.4 to -0.1 on a g BW^{-1} basis; Clark and Johnston, 1999). Factors shown to affect this relationship include lifestyle (e.g., pelagic vs. benthic; swimming style), temperature, and pH of the holding water (Killen et al., 2010; Ohlberger et al., 2012; Vaca and White, 2010). To understand the basis for these observed relationships, researchers have investigated the size-dependence of oxidative metabolism at the enzymatic level. In general, the mass-specific activities of oxidative enzymes in white skeletal muscle (e.g., citrate synthetase activity) scale negatively to body weight ($b \approx -0.25$), but scaling exponents differ substantially among species (Somero and Childress, 1980; Somero and Childress, 1990; Vetter and Lynn, 1997; Davies and Moyes, 2007; Moyes and Genge, 2010).

The purpose of this study was to evaluate the size-dependence of hepatic biotransformation in rainbow trout. Of interest was the question of whether fish size represents another factor responsible for observed differences in biotransformation rates within species. To perform this evaluation, we measured total cytochrome P450 (CYP) concentration and the activities of phase I and phase II metabolic enzymes in liver S9 fractions. Trout employed in this study were hatched from the same group of eggs and ranged in size by two orders of magnitude.

2. Materials and methods

2.1. Chemicals

The enzyme substrates benzo[*a*]pyrene (BAP), phenanthrene (PHEN), pyrene (PYR), 7-ethoxyresorufin (ER), *p*-nitrophenol (*p*-NP), and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich (St. Louis, MO). The purity of these chemicals was 95% or greater. The incubation cofactors uridine 5′-diphosphoglucuronic acid (UDPGA, > 98% pure), adenosine 3′-phosphate 5′-phosphosulfate (PAPS, > 60% pure), and reduced glutathione (GSH, > 98% pure) were also purchased from Sigma-Aldrich. β-nicotinamide-adenine dinucleotide phosphate (β-NADPH, > 95% pure) was purchased from Oriental Yeast Company (Tokyo, Japan). All other reagents, unless noted otherwise, were purchased from Sigma-Aldrich and were reagent grade or higher in quality.

2.2. Animals

2.2.1. Source and holding conditions

Rainbow trout (*Oncorhynchus mykiss*, Erwin strain) were obtained as fertilized embryos from the Upper Midwest Environmental Sciences Center (US Geological Survey, Lacrosse, WI) and grown to the sizes needed for this study. All fish originated from the same pool of embryos. The fish were held at 11 ± 1 °C under a 16 h light:8 h dark photoperiod and fed commercial trout food (Classic Trout; Skretting, USA, Tooele, UT). The feeding rate and particle size of the food were periodically adjusted to accommodate growth of the fish. Water used for fish holding was obtained directly from Lake Superior (single pass, sand filtered), and was sterilized by UV light before use. Chemical characteristics of the water were: total hardness, 45 to 46 mg L^{-1} as $CaCO_3$; alkalinity, 41 to 44 mg L^{-1} as $CaCO_3$; pH 7.6 to 7.8; total ammonia, < 1 mg L^{-1}; and dissolved oxygen, 85 to 100% of saturation. All trout holding conditions and experimental procedures were approved by an Institutional Animal Care and Use Committee in accordance with principles established by the Interagency Research Committee.

2.2.2. Selection of fish sizes

Our goal was to evaluate hepatic biotransformation in trout across the widest possible range of fish sizes, taking into account the minimum size required to provide enough tissue for measurement of enzyme activity and the need to avoid fish that were approaching sexual maturity. The minimum amount of pooled tissue needed to perform all planned assays was approximately 2 g. Preliminary experiments indicated that livers from 4 g trout could be cleared of blood and dissected from the body without the gall bladder attached. The number of 4 g animals needed to provide the required amount of S9 material was ~40 animals per pool, which was deemed to be acceptable.

The activities of hepatic phase I and phase II metabolic enzymes in trout change as they approach spawning condition, generally tending to increase, especially in males (Stegeman and Chevion, 1980; Koivusaari et al., 1981, 1984; Förlin and Haux, 1990). The strain of trout employed for this study spawns in the fall. Under the described holding conditions, these fish attain sexual maturity as they approach 3 yr of age, at which point they weigh 600–800 g. To minimize the effects of sexual maturation on enzyme activity, the largest animals were sampled in February, at approximately 2.3 yr of age. The sexual maturity of these animals was evaluated by weighing the gonads from each fish and calculating its gonadosomatic index (GSI; equal to the mass of gonads/body mass × 100).

Altogether, biotransformation was evaluated in four different sizes of fish. The weights of the animals were equally distributed on a $\log_{10}$ scale across two orders of magnitude, resulting in target body weights of 4, 20, 90, and 400 g. To accommodate individual differences in growth rate, an animal was accepted if its body weight was within 25% of the target value at the time of collection. A small number (< 10%) of 4 g animals were slightly larger.

2.3. Collection of livers and preparation of liver S9 fractions

The body weights of donor fish were routinely monitored by weighing subsets of fish drawn from the population of study animals. Livers were collected whenever the average weight for this sampled subset reached one of the target body weights. Five pools of liver tissue were collected for each size of fish.

S9 fractions were prepared from pooled livers following methods described by Johanning et al. (2012), modified as necessary for smaller animals. The fish were euthanized with an overdose of tricaine methanesulfonate (MS 222, 250 mg L^{-1}; Western Chemical Ind., Ferndale, WA) buffered with 750 mg L^{-1} $NaHCO_3$. Previous work has shown that MS 222 has no discernable impact on CYP protein concentrations or measured levels of CYP activity (Kleinow et al., 1986; Kolanczyk et al., 2003). All livers were cleared of blood in-situ by infusing with Hank's balanced salt solution (pH 7.80; ice-cold; free of $CaCl_2$, $MgSO_4$, and phenol red) containing 2.3 mM EDTA and 10 mM HEPES. In the larger fish (20, 90, and 450 g), clearing buffer was delivered through the hepatic portal vein using a hypodermic needle sized to fit the vessel. In the 4 g trout, this approach was not possible due to the small size of the hepatic portal vein. Instead, clearing buffer was administered by inserting a small needle (28 gauge, ½ inch) directly into the liver tissue. Once cleared, the liver was immediately excised from the body, leaving the gall bladder behind. The liver was then rinsed with a small volume of Hank's buffer to minimize possible contamination from bile. Livers were stored on ice in a small volume of homogenization buffer until all livers for a given pool were isolated. The livers were then blotted quickly and weighed. Due to their small size, livers from the 4 and 20 g fish were pooled before weighing. Livers from the 90 and 400 g fish were weighed individually. The livers were homogenized in 2 volumes of homogenization buffer using 4–5 strokes of a Potter-Elvehjem mortar and pestle. The homogenization buffer (pH 7.80 ± 0.05) consisted of 150 mM KCl, 50 mM Tris, 1 mM dithiothreitol, 2 mM EDTA and 250 mM sucrose. A small sample (250 μL) of this homogenate was collected to measure the recovery of microsomal enzymes (see Section