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Tissue Slice Technology for Assessing Alterations in Fish Hepatic Phase I and Phase II XME Activity

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ABSTRACT

Alterations to hepatic xenobiotic metabolizing enzymes (XMEs) is an important biomarker of contaminant exposure in aquatic toxicology. Measurement of XMEs in fish liver slices in vitro *is an emerging tool for examining enzyme activity and response within the intact 3-D architecture of the liver tissue. We examined integrated phase I/phase II, and phase II metabolism of XMEs from liver slices in control and B[a]P-treated rainbow trout and channel catfish. Fluorescent assay substrates to measure rates of metabolism included 7-methoxycoumarin (7-MC), 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC). Time-dependent increases in metabolism, and a lower rate of 7-MC metabolism compared with 7-EC metabolism, were observed at all time points for both fish species. In rainbow trout, B[a]P pretreatment caused a 10-fold increase in phase I metabolism of both 7-MC and 7-EC, and a 1.6-fold increase in phase II metabolism of 7-HC. Phase I activity in channel catfish was not notably altered by B[a]P pretreatment. However, B[a]P pretreatment in channel catfish caused a 48% decrease in phase II metabolism of 7-HC. These results indicate differences in baseline and B[a]P-altered XME profiles between rainbow trout and channel catfish.* © *1998 Elsevier Science Ltd. All rights reserved*

Fish cytochrome P450 (phase I) enzymes may be induced by organic compounds such as polyaromatic hydrocarbons, pesticides and coplanar polychlorinated biphenyls, and metals. For example, alteration in the activity of specific phase I enzymes, namely the CYP1A family, has been proposed as a means of monitoring environmental contamination (Goksøyr and Förlin, 1992; Stegeman *et al.*, 1992; Haasch *et al.*, 1993). However, many biochemical endpoints, including cytochrome P450 induction, appear relatively non-specific with regard to the inducing agent. To this end, we have examined species-specific differences in baseline and benzo[a]pyrene (B[a]P)-altered metabolic activity in

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hepatic tissue slices. Our approach utilized multiple alkoxycoumarin metabolic substrates (Haasch *et al.*, 1994) to examine differences in both phase I and phase II metabolism. These substrates permit rate measurements of *O*-demethylation, *O*-deethylation, glucuronidation and sulfation. Rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*), two species representing distinct ecological niches and important commercial fisheries, were chosen as models for this study.

Hepatic tissue slice technology has been adapted from mammalian models for use in fish metabolism studies (Kane and Thohan, 1996; Singh *et al.*, 1996). Precision-cut liver slices *in vitro* provide a source of xenobiotic metabolizing enzymes (XMEs) from within the functional, compartmentalized cellular architecture of the liver tissue (Azri *et al.*, 1990). An advantage of tissue slices, vs microsomes, as a source of XMEs includes the ability to measure integrated phase I and phase II metabolism without assay optimization. A mechanized slicing apparatus is used to produce uniformly thin liver slices (Krumdieck *et al.*, 1980). Coupled with dynamic tissue incubation, tissue slice technology allows exchange of gasses and nutrients during tissue incubation, resulting in enhanced metabolic and physiologic viability in culture.

Rainbow trout (*Oncorhynchus mykiss*, 260–320 g) and channel catfish (*Ictalurus punctatus*, 110–193 g) were laboratory acclimated at 12 and 20°C, respectively, for at least four weeks prior to treatment. Animals were dosed with 25 mg B[a]P kg^{-1}, I.P., dissolved in olive oil. Control fish were administered equivalent volumes of vehicle. Animals were euthanized with an overdose of buffered MS-222 and then cervically transected. Livers were harvested, weighed, and placed in ice-cold Krebs-Henseleit buffer. Livers cores were generated using a 10 mm stainless steel corer, and 250 μM slices were generated using a Krumdieck tissue slicer. Slices were incubated in dynamic organ culture in a modified Krebs-Henseleit buffer for up to 4 h at 22°C under ambient air conditions. Substrates for quantifying integrated phase I/II activity included 7-methoxycoumarin (7-MC, 100 μM) and 7-ethoxycoumarin (7-EC, 100 μM). Additionally, glucuronidation and sulfation, in the absence of phase I metabolism, were quantified using 7-hydroxycoumarin (7-HC, 100 μM). The presence of free (i.e. unconjugated) 7-HC metabolites from 7-EC and 7-MC, and hydrolyzed 7-HC glucuronide and sulfate conjugates were quantified spectrofluorometrically (Kane and Thohan, 1996) after 1, 2, and 4 h incubation. 7-HC was measured at an excitation wavelength of 370 nm and emission wavelength of 450 nm (Greenlee and Poland, 1977).

Time-dependent increases in metabolism, and a lower rate of 7-MC metabolism compared with 7-EC metabolism, was observed at all time points for both fish species. Phase II glucuronide and sulfate conjugates were detected using 7-MC, 7-EC and 7-HC substrates at all time points. In rainbow trout, B[a]P pretreatment caused a ten-fold increase in phase I metabolism of both 7-MC and 7-EC, and a 1.6-fold increase in phase II metabolism of 7-HC (Table 1). The increase in phase II metabolism primarily resulted from elevated glucuronidation. Based on the substrates used in this study, phase I activity in channel catfish was not significantly altered by B[a]P pretreatment. Interestingly, however, B[a]P pretreatment in channel catfish resulted in a 48% decrease in phase II metabolism of 7-HC.

These data indicate differences between rainbow trout and channel catfish in baseline and B[a]P-altered XME profiles. Further, these studies demonstrate relatively high sensitivity as well as versatility in metabolite quantification using precision-cut slices as a source of hepatic enzymes. Similar studies may also be conducted using extrahepatic tissues for evaluating a variety of XME activities.

TABLE 1
Activity Ratios (B[a]P treated : control) of Phase I and Phase II Xenobiotic Metabolizing Enzymes at 4 h (n = 3). Numbers < 1 Indicate Decreased Activities Compared with Controls

	O-Demethylation (7-MC)	*O-Deethylation (7-EC)*	*Glucuronidation (7-HC)*	*Sulfation (7-HC)*
Channel catfish	1.25	0.73	0.42	0.55
Rainbow trout	9.63	10.57	1.58	1.56

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