Chemosphere 150 (2016) 304–310

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Phase I metabolism of 3-methylindole, an environmental pollutant, by hepatic microsomes from carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*)

Vladimir Zlabek [a, *], Viktoriia Burkina [a], Francesc Borrisser-Pairó [b], Sidika Sakalli [a], Galia Zamaratskaia [a, c]

[a] University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic
[b] Product Quality Program, IRTA-Monells, Girona, Spain
[c] Swedish University of Agricultural Sciences, Uppsala, Sweden

HIGHLIGHTS

- metabolism of 3-methylindole (3MI, skatol) was described in fish.
- 3MI is metabolised in fish liver to 3MOI and I3C by CYP450.
- carp microsomes produced only one metabolite, 3MOI.
- rainbow trout microsomes produced both 3MOI and I3C.

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form
5 February 2016
Accepted 8 February 2016
Available online 23 February 2016

Handling Editor: David Volz

Keywords:
Hepatic microsomes
3-methylindole
3-hydroxy-3-metyloxindole
Indole-3-carbinol
Cytochrome P450

ABSTRACT

We studied the *in vitro* metabolism of 3-methylindole (3MI) in hepatic microsomes from fish. Hepatic microsomes from juvenile and adult carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) were included in the study. Incubation of 3MI with hepatic microsomes revealed the time-dependent formation of two major metabolites, 3-methyloxindole (3MOI) and indole-3-carbinol (I3C). The rate of 3MOI production was similar in both species at both ages. No differences in kinetic parameters were observed ($p = 0.799$ for V_{max}, and $p = 0.809$ for K_m). Production of I3C was detected only in the microsomes from rainbow trout. K_m values were similar in juvenile and adult fish ($p = 0.957$); V_{max} was higher in juvenile rainbow trout compared with adults ($p = 0.044$). In rainbow trout and carp, ellipticine reduced formation of 3MOI up to 53.2% and 81.9% and ketoconazole up to 65.8% and 91.3%, respectively. The formation of I3C was reduced by 53.7% and 51.5% in the presence of the inhibitors ellipticine and ketoconazole, respectively. These findings suggest that the CYP450 isoforms CYP1A and CYP3A are at least partly responsible for 3MI metabolism. In summary, 3MI is metabolised in fish liver to 3MOI and I3C by CYP450, and formation of these metabolites might be species-dependent.

Abbreviations: 2-AAP, 2-aminoacetophenone; 3MI, 3-methylindole; 3MOI, 3-methyloxindole; BFC, 7-benzyloxy-4-trifluoromethylcoumarin; BFCOD, 7-benzyloxy-4-trifluoromethylcoumarin O-debenzylation; CYP450, cytochrome P450; DAS, diallyl sulphide; ER, 7-ethoxyresorufin; EROD, 7-ethoxyresorufin O-deethylation; HFC, 7-hydroxy-4-trifluoromethylcoumarin; HMOI, 3-hydroxy-3-methyloxindole; I3C, indole-3-carbinol; KTZ, ketoconazole; NADPH, reduced β-nicotinamide adenine dinucleotide phosphate; PNP, p-nitrophenol; PNPH, p-nitrophenol hydroxylation.

* Corresponding author. University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, Vodnany, 389 25, Czech Republic.
E-mail addresses: vzlabek@frov.jcu.cz (V. Zlabek), vburkina@frov.jcu.cz (V. Burkina), francesc.borrisser@irta.cat (F. Borrisser-Pairó), sakalli@frov.jcu.cz (S. Sakalli), Galia.Zamaratskaia@slu.se (G. Zamaratskaia).

http://dx.doi.org/10.1016/j.chemosphere.2016.02.037
0045-6535/

1. Introduction

3-Methylindole (3MI), also known as skatole, is a naturally occurring substance found in mammalian faeces, cruciferous vegetables, beetroot and nectrandra wood. 3MI receives steady attention due to its wide range of biological effects (Hanafy and Bogan, 1982; Babol et al., 1998; Diaz and Squires, 2000). Previous studies have focused on the potential involvement of 3MI in several physiological processes in mammals. However, little attention was paid to the potential ecotoxicological consequences of this natural compound. The occurrence of 3MI in aquatic environments may be a cause for concern, mainly due to its significant load in wastewater. The presence of 3MI in aquatic environments is at least partly due to animal manure and anthropogenic waste. The discharge of untreated municipal wastewater during storm water overflow in heavy rain often delivers odorous volatile aromatic compounds such as 3MI to recipients. The surface runoff of liquid manure from livestock breeding facilities significantly contributes to pollution of surface water by 3MI. Improper application of liquid manure often used to fertilize agricultural fields increases risk of surface water contamination by this widely spread aromatic pollutant (Schüssler and Nitschke, 1999). Monitoring has indicated the presence of 3MI in contaminated groundwater (Smital et al., 2011; Yan et al., 2011; Gruchlik et al., 2013) and in the skin of fish from sites polluted by untreated wastewater (Schüssler and Nitschke, 1999).

3MI has been detected in wastewater at concentrations of 640–700 μg L^{-1} (Hwang et al., 1995) and up to 20 mg L^{-1} in decaying algal water environments (Peller et al., 2014). It is likely that 3MI accumulation in fish might be due to the degradation of endogenous amino acids.

The presence of this chemical in aquatic environments is of concern because of its eventual mutagenic properties (Thornton-Manning et al., 1991). To exert mutagenic effects, metabolic activation of 3MI by CYP450 is required. Numerous xenobiotic compounds that might negatively affect physiological processes in fish include substrates of cytochrome P450 (CYP450). The metabolism of 3MI has been predominantly studied in mammalian species, particularly in pigs due to its negative effects on meat quality (Zamaratskaia and Squires, 2009), and in ruminants due to pneumotoxicity (Yost, 1989). Hepatic skatole metabolites formed during phase-I metabolism in mammals include 2-aminoacetophenone (2-AAP), 3-methyloxindole (3MOI), 5-hydroxyskatole, 6-hydroxyskatole, 3-hydroxy-3-methylindolenine, 3-hydroxy-3-methyloxindole (HMOI) and indole-3-carbinol (I3C) (Diaz et al., 1999). Currently, no information is available about the metabolism of 3MI by fish. However, the induction of hepatic CYP1A by dietary I3C was previously studied in rainbow trout (Takahashi et al., 1995). It is essential to fully understand the metabolic pathways of 3MI to evaluate its eventual toxicity to fish.

Common carp and rainbow trout are economically important fish species. Cytochrome P450 enzymes involved in xenobiotic metabolism are well characterised in these species (Rabergh et al., 2000; Whyte et al., 2000; Lee et al., 2001; Fisher et al., 2006; Uno et al., 2012). Additionally, both common carp and rainbow trout were used as test organisms in toxicology tests, environmental monitoring and nutrition studies (Gaillard et al., 2014; Salze et al., 2014).

In the present study, we investigated 3MI metabolism by fish hepatic microsomes. For this purpose, we used hepatic microsomes from two fish species, carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), at two ages.

2. Materials and methods

2.1. Chemicals and reagents

3MOI, I3C, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), ellipticine, ketoconazole (KTZ), diallyl sulphide (DAS), resorufin, 7-ethoxyresorufin (ER), 7-hydroxy-4-trifluoromethylcoumarin (HFC), 7-benzyloxy-4-trifluoromethylcoumarin (BFC), p-nitrophenol (PNP), 4-nitrocatechol, and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (Steinheim, Germany). HMOI was synthesised as described elsewhere (England et al., 2007). HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany).

2.2. Fish

The juvenile and adult rainbow trout and carp were purchased from a local fishery (Czech Republic). The fish were transferred to aquaria containing 250 L of freshwater for a two weeks acclimatization period. The freshwater conditions were chosen according to water source in the fishery (temperature 14 ± 1 °C, pH 7.7 ± 0.1, dissolved oxygen 8.5–9.0 mg L^{-1}). The fish were not fed for 24 h prior to sampling to avoid prandial effects during the assay. The juvenile and adult rainbow trout and carp (total n = 32, 8 individuals per group) were measured [(mean total length ± standard deviation (S.D.)]: 217.6 ± 5.6 mm, 318.1 ± 20.69 mm, 226.1 ± 24.7 mm and 490 ± 19.4 mm and weighed (mean weight ± S.D.): 139.3 ± 10.2 g, 368.9 ± 35.78 g, 175.1 ± 54.4 g and 2356 ± 31.4 g, respectively. Fish age 1, 2, and 1, 4 years corresponded to juvenile and adult rainbow trout and carp, respectively. Fish were handled according to national and institutional guidelines for the protection of human subjects and animal welfare, the unit is licensed (No. 53100/2013-MZE-17214) according to Czech National Directive (Law against Animal Cruelty, No. 246/1992). Before sampling procedure, fish were anesthetized in an ice bath and immediately cut of the spinal cord. The fish were dissected and liver samples (without gall bladders) were quickly removed and immediately frozen and stored at −80 °C until use for preparation of microsomal fractions.

2.3. Preparation of microsomal fraction and protein measurement

The microsomal fraction was prepared from liver homogenate by differential centrifugation (Burkina et al., 2012). The levels of microsomal protein were estimated by bicinchoninic acid assay using bovine serum albumin as a standard (Smith et al., 1985). The samples were then diluted to a protein content of 10 mg mL^{-1}.

Twelve pooled microsomal fractions from either 2 or 3 individual fish in each were prepared from carp (3 pools from juvenile and 3 from adult fish) and rainbow trout (3 pools from juvenile and 3 from adult fish) and stored at −80 °C until analysis.

Males and females were not equally represented in pools. Sex identification was not possible in all juvenile fish. In adult fish there was lack of male fish therefore only one male was included in each microsomal pool.

2.4. Formation of skatole metabolites

The concentrations of 3MI and its metabolite in the incubations were determined by interpolation of multilevel standard curves made with authentic standards. Metabolites formation was measured as previously described for pigs (Diaz et al., 1999; Borrisser-Pairo et al., 2015), with slight modifications. All