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Effects of waterborne nitrite on phase I–II biotransformation in channel catfish (*Ictalurus punctatus*)

J.F. González [a], P.L. Del Valle [b], S. Thohan [c], A.S. Kane [b,*]

[a] *Universidad Nacional de Colombia, School of Veterinary Medicine, Bogotá, Colombia, South America*
[b] *Department of Veterinary Medicine, Aquatic Pathobiology Center, University of Maryland, 8075 Greenmead Drive, College Park, MD 20742, USA*
[c] *Covance Laboratories, Inc., Division of Metabolic Chemistry, Madison, WI 53707, USA*

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Abstract

The effects of waterborne nitrite (3 mg/l NO_2) on channel catfish were studied to evaluate changes in hematological parameters and phase I–II biotransformation in liver slices. Nitrite-exposed fish had significantly higher methemoglobin, blood and liver nitrite, and significantly lower pO_2 than control fish. Total phase I-mediated metabolism of 7-ethoxycoumarin (EC) was not altered in nitrite-exposed fish compared with control fish (291±43 and 312±20 pmol/mg/h, respectively). However, phase II glucuronosyltransferase-mediated metabolism of 7-hydroxycoumarin (HC), both as a phase I metabolite of EC and as a parent substrate, was elevated in nitrite-exposed fish (204±17 and 1007±103 pmol/mg/h, respectively) as compared to control fish (149±14 and 735±87 pmol/mg/h) ($P < 0.05$). Sulfotransferase-mediated metabolism of HC (as a metabolite of EC and as a parent substrate) was not notably altered in nitrite-exposed fish (95±16 and 617±33 pmol/mg protein/h, respectively) as compared with control fish (118±24 and 575±55 pmol/mg/h, respectively). These studies indicate that in vivo nitrite exposure and associated changes in hematological parameters do not appear to affect hepatic phase I EC biotransformation in channel catfish. However, subtle but significant changes in phase II glucuronidation, but not sulfation activity, were observed. The mechanism of these alterations is unclear. However, the data suggest that environmentally realistic concentrations of nitrite may affect the dynamics of conjugative metabolism in exposed fish.

Keywords: Nitrite; Biotransformation; Channel catfish; Xenobiotic metabolism

* Corresponding author. Tel.: +1-301-935-6083, ext. 100; fax: +1-301-935-6079.
E-mail address: akane@umaryland.edu (A.S. Kane).

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Effects of suboptimal water quality, particularly elevated nitrite (NO_2), have long received attention due to their adverse effects on freshwater fish health and aquacultural mortalities (Doblande & Lackner, 1996; Margiocco et al., 1983; Smith & Williams, 1974). Nitrite poisoning reduces the oxygen-carrying capacity of the blood by inducing methemaglobinemia and, under in vitro hypoxic conditions, has been linked to inhibition of phase I (cytochrome P450-mediated) biotransformation in rats, rabbits and fish (Arillo, Mensi & Pirozzi, 1984; Kahl, Wulff & Netter, 1978). However, there have been no previous investigations with fish to examine the effects of in vivo NO_2 exposure on either phase I or phase II metabolism. The present study was designed to test the hypothesis that in vivo NO_2 exposure, with its associated changes in blood chemistry, has no effect on phase I or phase II biotransformation.

Healthy, laboratory-acclimated channel catfish were exposed to 0.0 and 3.0 mg/l NO_2 (from $NaNO_2$) in 500-l glass aquaria with daily static renewal and gentle aeration. Aqueous nitrite concentrations were monitored spectrophotometrically, and blood and liver nitrite concentrations were determined using a micromethod (Shechter, Gruener & Shuval, 1972). Preliminary blood chemistry data from 10 fish indicated that methemoglobin and blood and liver nitrite were higher at 96 h compared with fish exposed for 24 and 48 h (González, 1997). Therefore, analyses of phase I–II xenobiotic metabolizing enzyme activities and blood chemistry were performed on fish after exposure to NO_2 for 96 h.

Blood samples were analyzed for hematocrit, plasma proteins, hemoglobin, methemaglobin, and pO_2 using an automated oxymeter. Liver slices from exposed animals were individually incubated with either 7-ethoxycoumarin (EC, 100 μM) or 7-hydroxycoumarin (HC, 100 μM) substrates in dynamic organ culture and phase I and phase II metabolites from EC and phase II metabolites from HC were extracted and determined spectrofluorometrically (Kane & Thohan, 1996; Kane, Thohan & Weiner, 1998).

Nitrite-exposed fish had significantly higher methemoglobin, blood and liver nitrite, and significantly lower pO_2 than control fish (Table 1). Hematocrit, plasma proteins and hemoglobin values between nitrite-exposed fish and control fish were not significantly different. Total phase I mediated metabolism of 7-EC was not

Table 1
Blood chemistry data from 96-h control and nitrite-exposed channel catfish (data are means±S.E.M.; $n = 10$–12 for each group)

Variable	Control fish	NO_2-exposed fish	Significance[a]
Hematocrit (%)	31.2±2.5	33.0±1.96	0.57
Plasma protein (g/dl)	4.1±0.23	4.14±0.13	0.93
Hemoglobin (g/dl)	6.0±0.55)	6.38±0.61	0.57
Methemoglobin (g/dl)	0.66±0.16	45.54±4.75	0.000001*
Blood nitrite (μg/ml)	0.10[b]	3.72±1.12	0.01*
Liver nitrite (μg/ml)	0.10[b]	1.01±0.37	0.02*
pO_2 (mm Hg)	39.1±8.53	19.77±2.25	0.05*

[a] Probability of type II error based on *t*-test; asterisk indicates significant difference.
[b] Data below detection limit of 0.10 (detection limit used to make statistical inference).

altered in nitrite-exposed fish compared with control fish (291±43 and 312±20 pmol/mg/h, respectively). However, phase II glucuronosyltransferase mediated metabolism of 7-HC, both as a phase I metabolite of EC and as a parent substrate, was elevated in nitrite-exposed fish (204±17 and 1007±103 pmol/mg/h, respectively) as compared to control fish (149±14 and 735±87 pmol/mg/h) ($P < 0.05$) (Fig. 1). Sulfotransferase-mediated metabolism of HC (as a metabolite of EC and as a parent substrate) was not notably altered in nitrite-exposed fish (95±16 and 617±33 pmol/mg protein/h, respectively) as compared with control fish (118±24 and 575±55 pmol/mg/h, respectively).

These studies indicate that hepatic phase I biotransformation of EC in channel catfish does not appear to be affected by in vivo exposure to 3 mg/l nitrite or changes in hematological parameters associated with the nitrite exposure. Further, the 'coupling' of phase I to phase II metabolic pathways using EC as parent substrate was similarly unaffected as indicated by the remaining amount of unconjugated free HC

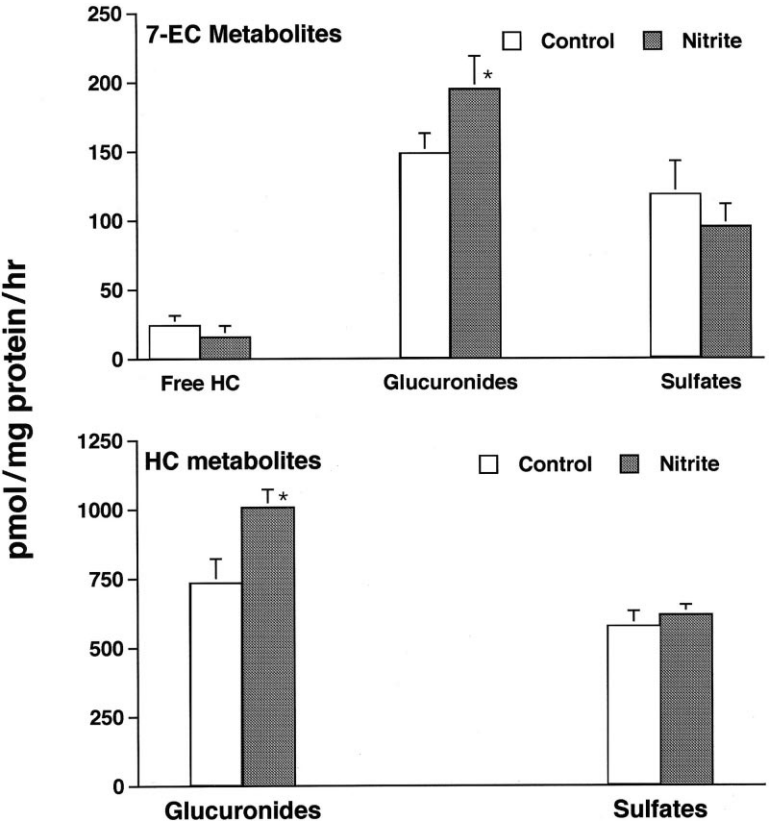

Fig. 1. Top panel: integrated phase I and phase II metabolism of 7-ethoxycoumarin (EC; phase I metabolites include free 7-hydroxycoumarin (HC), glucuronide and sulfate conjugates). Bottom panel: phase II metabolism of 7-HC as parent substrate (phase II metabolites include glucuronide and sulfate conjugates). Data are means±S.E.M.; $n = 5$–6; asterisk indicates significant difference at $\alpha = 0.05$.

(Fig. 1, top). However, subtle but significant changes in phase II glucuronidation, but not sulfation activity were observed. Although hematological values in control and nitrite-exposed fish were within the range of previously reported values (Huey, Simco & Criswell, 1980; Tomasso, Simco & Davis, 1979; Tucker, 1983; Tucker, Francis-Floyd & Beleau, 1989), the increased methemoglobin and decreased pO_2 observed in the nitrite-exposed fish in vivo were not sufficient to inhibit phase I biotransformation as indicated by earlier efforts under hypoxic conditions in vitro (Arillo et al., 1984; Kahl et al., 1978). The mechanism of the phase II alterations is unclear. However, factors that may be involved in these changes include direct effects of NO_2, reduced pO_2 on the enzyme system, and reduced accessibility of cofactor. Nitrite stress may also increase cortisol (Tomasso, Davis & Simco, 1981) and other steriod moieties as part of the general adaptation syndrome; the glucuronidation pathway may be preferentially involved in the metabolism of these steriods in channel catfish. The data suggest that environmentally realistic concentrations of nitrite may affect the dynamics of conjugative metabolism in exposed fish.

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