Effects of waterborne nitrite on phase I–II biotransformation in channel catfish

(Ictalurus punctatus)

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Abstract

The effects of waterborne nitrite (3 mg/l NO2) 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 pO2 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 compared with control 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). Sulphotransferase-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
Effects of suboptimal water quality, particularly elevated nitrite (NO₂), 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 methemoglobinemia 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 NO₂ exposure on either phase I or phase II metabolism. The present study was designed to test the hypothesis that in vivo NO₂ 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 NO₂ (from NaNO₂) 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 NO₂ for 96 h.

Blood samples were analyzed for hematocrit, plasma proteins, hemoglobin, methemoglobin, and pO₂ 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 pO₂ 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>
<thead>
<tr>
<th>Variable</th>
<th>Control fish</th>
<th>NO₂-exposed fish</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>31.2±2.5</td>
<td>33.0±1.96</td>
<td>0.57</td>
</tr>
<tr>
<td>Plasma protein (g/dl)</td>
<td>4.1±0.23</td>
<td>4.14±0.13</td>
<td>0.93</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>6.0±0.55</td>
<td>6.38±0.61</td>
<td>0.57</td>
</tr>
<tr>
<td>Methemoglobin (g/dl)</td>
<td>0.66±0.16</td>
<td>45.54±4.75</td>
<td>0.000001*</td>
</tr>
<tr>
<td>Blood nitrite (µg/ml)</td>
<td>0.10b</td>
<td>3.72±1.12</td>
<td>0.01*</td>
</tr>
<tr>
<td>Liver nitrite (µg/ml)</td>
<td>0.10b</td>
<td>1.01±0.37</td>
<td>0.02*</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>39.1±8.53</td>
<td>19.77±2.25</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

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

![Graph](image-url)

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 pO2 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 NO2, reduced pO2 on the enzyme system, and reduced accessibility of cofactor. Nitrite stress may also increase cortisol (Tomasso, Davis & Simco, 1981) and other steroid moieties as part of the general adaptation syndrome; the glucuronidation pathway may be preferentially involved in the metabolism of these steroids in channel catfish. The data suggest that environmentally realistic concentrations of nitrite may affect the dynamics of conjugative metabolism in exposed fish.

References


