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B[a]P–DNA binding in early life-stages of Atlantic tomcod: population differences and chromium modulation

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Abstract

Atlantic tomcod (*Microgadus tomcod*) from the Hudson River (HR) are resistant at the molecular and organismic levels to the effects of exposure to dioxin-like aromatic hydrocarbon (AH) compounds, but much less so to benzo[a]pyrene (B[a]P). The aims of this study were to determine in early life-stages of tomcod exposed to B[a]P: (1) if DNA binding levels differed between fish from the HR and Miramichi River (MR), and (2) if co-exposure to chromium could modulate this genotoxic effect. After exposure to [^{3}H]B[a]P alone, DNA-bound radioactivity was 5–10-fold higher in embryos and larvae of MR than HR descent. Co-exposure to chromium modulated DNA binding levels in offspring of both populations. In MR embryos, co-exposure to chromium inhibited B[a]P uptake. These results demonstrated resistance to the genotoxic effects of B[a]P in early life stages of HR tomcod at an ecologically important endpoint and suggest the ability of chromium to modulate AH-induced genotoxicity.

Keywords: Resistance; Genotoxicity; Early life-stages; Hydrocarbons; Metal–organic interactions; DNA-binding

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Larval Atlantic tomcod (*Microgadus tomcod*) from the Hudson River (HR) are resistant to CYP1A1 mRNA induction (Roy, Courtenay, Yuan, Ikonomou, & Wirgin, 2001) and embryo-larval toxicity by PCBs and TCDD, but much less so to PAHs such as B[a]P (Chambers and Wirgin, unpublished data). The HR is contaminated by aromatic hydrocarbons (AHs) and other pollutants, including metals known to modulate in vitro CYP1A1 inducibility via the AH receptor pathway (Bruschweiler, Wurgler, & Fent, 1996; Maier, Dalton, & Puga, 2000). Acute in vivo co-exposure to cadmium, nickel, chromium, and arsenic inhibited hepatic CYP1A1 mRNA expression induced by AHs in adult tomcod (Sorrentino, Roy, Chambers, Courtenay, & Wirgin, 2001). B[a]P is metabolized by CYP1A1 to form DNA adducts and the resulting DNA damage may play a role in early life-stage toxicity. Our goals were to determine in early life-stages of tomcod: (1) if levels of DNA adducts differed between HR and MR fish exposed to B[a]P; and (2) if co-exposure to chromium could modulate this genotoxic effect.

Adult tomcod were collected from the HR and Miramichi River (MR) and mated (Roy et al., 2002). Two groups of 20 embryos each of HR and MR ancestry were exposed waterborne (10 μl/ml) for 48 h to the following environmentally relevant concentrations of B[a]P and chromium; 1, 5, or 10 ppb [^{3}H]B[a]P (Amersham Biosciences; 307 mCi/mg), combinations of 10 ppb [^{3}H]B[a]P and 0.1, 1, or 10 ppm K_2CrO_4, and to an equal volume of acetone vehicle. DNA samples were isolated by incubation of embryos in NH_4OH/Triton X-100 buffer, phenol/chloroform extractions, and ethanol precipitations (Wirgin, D'Amore, Grunwald, Goldman, & Garte, 1990). DNA concentrations and purities were evaluated by UV spectrophotometry at 260 and 280 nm. Five ml of Ecoscint (National Diagnostics) was added to each sample and radioactivity was measured by liquid scintillation counting.

From each population, 16 post-yolk-sac larvae were exposed for 48 h to 5 ppb [^{3}H]B[a]P alone, 5 ppb [^{3}H]B[a]P in combination with 0.1 ppm K_2CrO_4, and to acetone. DNA was extracted as described above from two pools of 5 larvae from each population.

The effect of co-exposure to chromium on B[a]P uptake was determined in groups of 20 embryos of MR descent exposed for 48 h to 10 ppb [^{3}H]B[a]P alone or in combination with 0.1, 1, and 10 ppm K_2CrO_4, and to acetone. Uptake was also measured in duplicate pools of 2–3 larvae from each population after exposure to 5 ppb [^{3}H]B[a]P alone or in combination with 0.1 ppm K_2CrO_4. After 48 h, embryos and larvae were extensively washed in H_20, incubated for 4 h in 2 ml of Soluene (Packard) at 50 °C, cooled, 10 ml of Hionic Fluor (Packard) was added, and radioactivity was determined.

No mortality was observed in either embryos or larvae following treatments. Exposure of HR embryos to 1, 5, and 10 ppb [^{3}H]B[a]P resulted in 3.6, 4.1, and 26.4 cpm/μg DNA, respectively. In MR embryos, the levels of DNA-bound radioactivity were much higher at all three levels of B[a]P exposure: 8.6, 89.6, and 136.6 cpm/μg DNA, respectively (Fig. 1(a)). In embryos from both populations, co-exposure to 0.1 ppm K_2CrO_4 decreased levels of DNA-bound radioactivity (Fig. 1(b)). Modulation of DNA-binding levels was inversely related to K_2CrO_4 concentration: in HR embryos the DNA-bound radioactivity was −35.0%; −12.7%, and +5.9% of that in

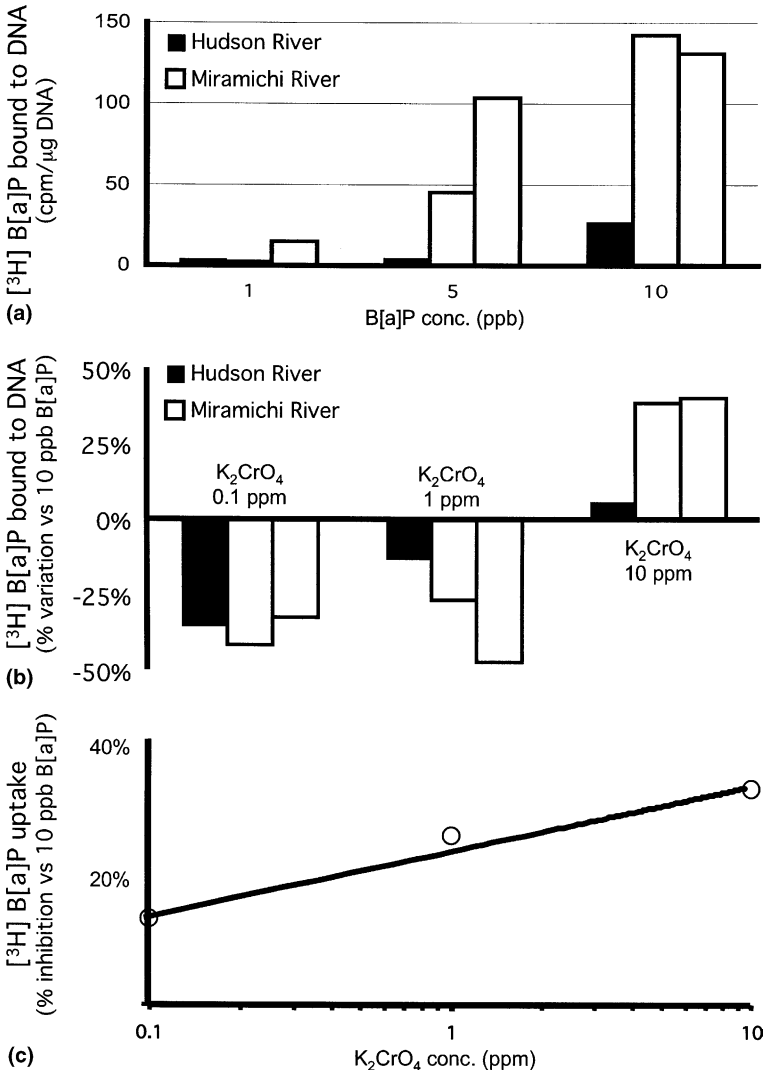

Fig. 1. Effects of population origin and co-exposure to chromium on B[a]P–DNA binding and uptake of B[a]P in tomcod embryos. Panel A: levels of [^{3}H]B[a]P bound to DNA in Hudson River (HR) and Miramichi River (MR) embryos waterborne exposed to 1, 5, and 10 ppb B[a]P for 48 h. Panel B: effects of co-exposure to 10 ppb B[a]P and 0.1, 1, and 10 ppm K_2CrO_4 on [^{3}H]B[a]P binding to DNA in HR and MR tomcod embryos. Panel C: effect of co-exposure to graded concentrations of K_2CrO_4 (0.1, 1, and 10 ppm) on the uptake of 10 ppb [^{3}H]B[a]P in MR tomcod embryos. Results are expressed as percentage of variation versus B[a]P alone, after normalizing the cpm for differences in total weight of embryos.

embryos exposed to 10 ppb B[a]P alone and in MR embryos −37.2%, −36.5%, and +39.7% at 0.1, 1, and 10 ppm K_2CrO_4. After 48 h exposure, the uptake of [^{3}H]B[a]P by MR embryos was inhibited by co-exposure to chromium (Fig. 1(c)): 0.1, 1, and 10 ppm K_2CrO_4 reduced B[a]P uptake by 13.2%, 25.7%, and 32.8%, respectively.

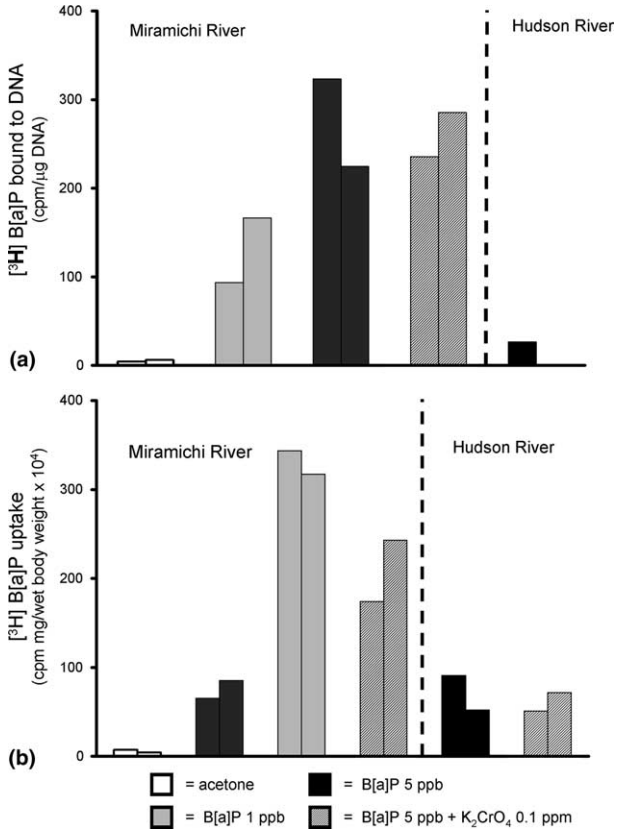

Fig. 2. Effects of population origin and co-exposure to K_2CrO_4 on B[a]P–DNA binding and uptake of B[a]P in tomcod post-yolk-sac larvae. Panel A: comparison of [^{3}H]B[a]P bound to DNA in tomcod larvae exposed for 48 h to waterborne B[a]P alone or in combination with K_2CrO_4 (MR only). MR larvae were exposed to 1 and 5 ppb B[a]P alone or to 5 ppb B[a]P in combination with 0.1 ppm K_2CrO_4 and HR to 5 ppb B[a]P alone. Panel B: comparison of B[a]P uptake in MR exposed to 1 and 5 ppb [^{3}H]B[a]P and HR exposed to 5 ppb [^{3}H]B[a]P and to a combination of 5 ppb [^{3}H]B[a]P and 0.1 ppm chromium.

Results obtained with larvae were similar. DNA-bound radioactivity was 10 times lower in HR compared to MR larvae exposed to 5 ppb [^{3}H]B[a]P (Fig. 2(a)). This effect was only partly related to differences in the uptake of B[a]P because the radioactivity in MR larvae was 4.6-fold higher than that in HR larvae (Fig. 2(b)). In MR larvae, co-exposure to 0.1 ppm K_2CrO_4 did not affect levels of B[a]P binding to DNA (−5%), whereas it decreased the uptake by 37%. For HR larvae, data are available for uptake only and showed a 14% decrease following K_2CrO_4 co-exposure.

We have demonstrated that B[a]P-exposed embryos and larvae from a population sensitive to AHs show higher DNA-binding levels than early life-stages from a resistant population. Thus, response at a potentially important genotoxic endpoint, DNA binding of B[a]P, was consistent with earlier results that showed reduced

CYP1A1 mRNA inducibility (Courtenay et al., 1999; Roy et al., 2001) and teratogenicity (Chambers, Whitting, Cerino, & Wirgin, 2003) in tomcod from the HR compared to the MR. After a 48 h exposure to graded doses of [^{3}H]B[a]P, DNA-binding levels were approximately 5-fold less in HR embryos than in embryos of MR ancestry. In larvae, the difference was even greater, with MR larvae being 10-fold more sensitive than those of HR descent. One possible explanation for these effects is the reduced CYP1A1 inducibility in the HR population. Similarly, adult *Fundulus heteroclitus* from a population resistant to dioxin-like compounds were protected from the formation of B[a]P-induced hepatic DNA adducts (Nacci, Kohan, Pelletier, & George, 2002). This is also the first report of HR tomcod exhibiting resistance to B[a]P-induced toxicity.

In embryos from both populations, the effect of co-exposure to chromium had opposite effects: low concentrations decreased levels of B[a]P–DNA binding, while high concentrations increased it. This result is at least in part consistent with the effects of acute co-exposures to B[a]P and chromium on hepatic CYP1A1 mRNA inducibility in adult tomcod where co-exposure resulted in lower expression of CYP1A1 mRNA than in fish treated with B[a]P alone (Sorrentino et al., 2001). Furthermore, the inhibition of uptake of B[a]P in embryos by chromium suggests a mechanism through which co-exposure to chromium may alter the biological effects of exposure to B[a]P. However, alteration of uptake cannot solely be responsible for the difference in DNA binding. The uptake of B[a]P after a 48 h exposure decreased with increasing concentrations of chromium, whereas the effects of chromium on B[a]P–DNA binding showed an opposite trend.

These results highlight the importance of the study of the biological effects of mixtures compared to those of individual pollutants to better understand the processes affecting populations from chemically impacted ecosystems.

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