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Short communication

Heme oxygenase induction and biliverdin excretion: Implications for the bile fluorescence biomarker

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Abstract

The measurement of bile fluorescence has become a popular biomarker to demonstrate the exposure of fish to polycyclic aromatic hydrocarbons. Conflicting data have been published on how to normalize bile fluorescence. To investigate if normalization to biliverdin is a suitable method, experiments were performed to study the mechanisms related to biliverdin excretion in fish. Channel catfish (*Ictalurus punctatus*) were dosed with mixtures of benzo[*a*]pyrene and cadmium, chlorinated phenols or borneol. The results showed that under increasing toxicant stress, more biliverdin was excreted per amount of protein. To investigate if the increased biliverdin excretion was related to increased heme degradation, enzymatic activity of heme oxygenase (HO) was measured in liver homogenates. The fish dosed with chemical mixtures had significantly higher HO activity than the control fish, and a significant correlation was observed between HO activity and biliverdin concentration in the bile. It is concluded that chemical mixtures of environmental pollutants can induce HO activity and that this chemical stress leads to increased biliverdin excretion. The elucidation of this mechanistic pathway warrants that bile fluorescence is better expressed per amount of bile protein than per biliverdin absorption.

Keywords: Bile fluorescence; PAH metabolites; Biliverdin; Heme oxygenase

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The release of polycyclic aromatic hydrocarbons (PAHs) into the environment has been recognized as a serious environmental problem for decades (Varanasi, 1989). Several biomarkers have been developed to measure the exposure and potential effects of PAHs in wildlife and aquatic organisms (Van der Oost et al., 2003). Induction of the cytochrome P450-1A enzyme has been widely recognized as a sensitive biochemical biomarker. Another biomarker for PAH exposure is the measurement of PAH metabolites in bile. Aromatic hydrocarbons, including their metabolites, are highly fluorescent, and can easily be measured in bile samples (Lin et al., 1996; Aas et al., 2000). A problem with analyzing bile samples is the normalization of the fluorescence data (Aas et al., 2000). Three methods have been used: fluorescence units per bile volume, units per mg protein in bile, and units per amount of biliverdin in bile. Bile volume is highly variable, depending on feeding rates. Under reduced feeding regimes bile will undergo concentration in the gall bladder, which makes this method unreliable (Richardson et al., 2004). Expression of fluorescence in relation to the amount of biliverdin in bile has been investigated, but led to contradictory results (Aas et al., 2000; Ruddock et al., 2003). So far the approach to solving this normalization problem has been pragmatic: which method reduces the variability of the data the most. The objective of this study was to evaluate a method based on a mechanistic approach: what processes regulate biliverdin excretion, and do they justify normalization of fluorescence to biliverdin amount in bile.

Channel catfish (*Ictalurus punctatus*, average 240 mm, 150 g) were dosed (i.p.) with benzo[*a*]pyrene (10 mg/kg) alone or in combination with pentachlorophenol, trichlorophenol and borneol (each 25 mg/kg). Livers and gall bladders were sampled on days 2, 3 and 6, sample size was 3 fish per treatment per sampling day. Bile was dissolved in 1 ml acetate buffer (0.1 M, pH 5.0), centrifuged, and analyzed for protein content. Biliverdin absorption was measured at 365 nm in a 1:5 dilution in methanol/water (50:50). Benzo[*a*]pyrene fluorescence was measured in 1:1000 diluted samples at 380/430 nm. Heme oxygenase activity in liver was measured in reaction mixtures consisting of 0.5 g microsomal protein, 2 mM glucose-6-phosphate, 1 mM NADPH, 1 unit of glucose-6-phosphate dehydrogenase, and 2.5 mM hemin, all in 100 μl of 0.25 M sucrose/20 mM Tris–HCl buffer of pH 7.4 (Ryter et al., 1999). After 20 min the reaction was stopped with ethanol/dimethylsulfoxide (95:5, v:v), containing 1 μM mesoporphyrin as internal standard. The enzymatic degradation of hemin was analyzed by gradient HPLC on a C18 column and a UV–Vis detector at 405 nm.

Injection of BaP caused a large increase of bile fluorescence. While fluorescence in control samples was almost undetectable, a dose of 10 mg/kg of BaP resulted in a 50-fold increase of fluorescence after 2 days. When BaP was dosed together with the other toxic compounds, the biliary excretion of BaP metabolites was increased an additional 2–4-fold. The same process was observed when rainbow trout were dosed with combinations of dieldrin and BaP (Barnhill et al., 2003) and is a result of co-induction of BaP-metabolizing enzymes.

Bile fluorescence was expressed as fluorescence per mg bile protein and as fluorescence per amount of biliverdin. If both methods were independent, they should result in a linear correlation when plotted against each other. Fig. 1 demonstrates that a linear regression line is not the best fitting model. The plotted line is a hyperbolic model with excellent goodness of fit ($R^2 = 0.97$). This indicates that fluorescence expressed per amount of biliverdin is saturating with increasing fluorescence. This can only be explained by a concurrent increase of bile fluorescence and biliverdin. Further analysis revealed that within each