Benzo(a)pyrene Induces CYP1A and p53 Expression in Brown Bullhead (*Ameiurus nebulosus*)

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Abstract

Exposure to the environmental contaminant benzo(a)pyrene (BaP) increases health risks. This study investigated the effect of BaP on the expression of cytochrome P450 1A (CYP1A) and tumor suppressor protein p53 in brown bullhead (Ameiurus nebulosus). Fish were exposed to three different doses of BaP (0 mg, 6 × 10–6 µg, and 0.225 mg) every other day for a week, and liver and gill tissues were collected for western blot analysis of CYP1A and p53 protein levels. Results showed that high-dose BaP (0.225 mg) exposure significantly upregulated CYP1A protein in both liver and gill tissues (21.291X and 7.692X, respectively), indicating induction of a known detoxification system. In contrast, high-dose BaP exposure (0.225 mg) only upregulated p53 protein expression in the gills (35.38X), suggesting tissue-specific effects of BaP. These findings suggest that brown bullhead can serve as a model for screening the effect of biological exposure to BaP. Additionally, the study also observed an increase in tricaine methane sulfonate (MS-222) tolerance in fish exposed to high doses of BaP, which may be attributed to increased CYP1A expression and detoxification capacity. Overall, this study provides insight into the mechanism of BaP toxicity, CYP1A and p53 response, and further validates the importance of monitoring its environmental levels and potential impacts on aquatic ecosystems.

CYP1A Background

- CYP1A, among cytochrome P450 (CYP) isoforms, mainly contributes to the metabolism of drugs, environmental pollutants, and physiological substances (Almazroo et al., 2017).
- Following the aryl hydrocarbon receptor (AHR) signaling pathway, the released CYP1A reacts with xenobiotics, forming a water-soluble product that can be dispelled from the body (Monostory et al., 2009). The intermediate BaP-7,8-epoxide can be easily removed from the body (Moserová et al., 2009).
- Although CYP1A removes toxins, it can also metabolize toxins to their mutagenic form, BPDE (Stading et al., 2020). BPDE can only be removed if the DNA adduct is removed through nucleotide excision repair. However, if the DNA adduct fails to be removed, BPDE can form malignant tumors.
- Since reactive epoxides can be more dangerous than the original compound, the activation of CYP1A1 can lead to BaP-mediated liver toxicity and death (Uno et al., 2001). In this worse scenario, the body employs p53, a tumor suppressor, to prevent cancer.

p53 Background

As a tumor suppressor gene, p53 regulates cell division, proliferation, and programmed cell death (apoptosis). When DNA becomes damaged, p53 binds to the DNA and determines whether the DNA should be repaired or removed. If the DNA can be repaired, this protein activates other proteins to repair the damage. Since BaP damages the DNA, p53 becomes activated and signals for DNA repair, growth arrest, or apoptosis (Yuan et al., 2017). If the DNA must be removed, p53 stimulates the production of p21, which interacts with a cell division-stimulating protein cyclin-dependent kinase 2 (cdk2), to prevent cell division and signal apoptosis (Surget et al., 2014; Matlashewski et al., 1984).

Conclusion

Western blot analysis revealed a significant increase in CYP1A protein levels in fish exposed to high doses of BaP, presenting consistent results with the role of CYP1A as a crucial protein involved in the metabolism of xenobiotics. BaP induced p53 expression only in the gills of brown bullhead but not in the liver, suggesting tissue-specific effects of BaP. Additionally, the tolerance of tricaine methane sulfonate (MS-222) increased in fish that were exposed to high doses of BaP, suggesting the correlation with the enhanced expression of CYP1A and its increased detoxification ability.

Future Direction

- CYP1A can remove toxins but also create a more toxic product. Therefore, further studies are required to determine the long-term effects of the upregulated CYP1A gene.
- p53 is only induced in the gills but not in the liver. Therefore, future studies can compare the BaP-induced p53 expression in different tissues.
- However, no studies have yet investigated the relationship between CYP1A and MS-222. Further research is needed to elucidate this interaction and its potential effects.

Methods

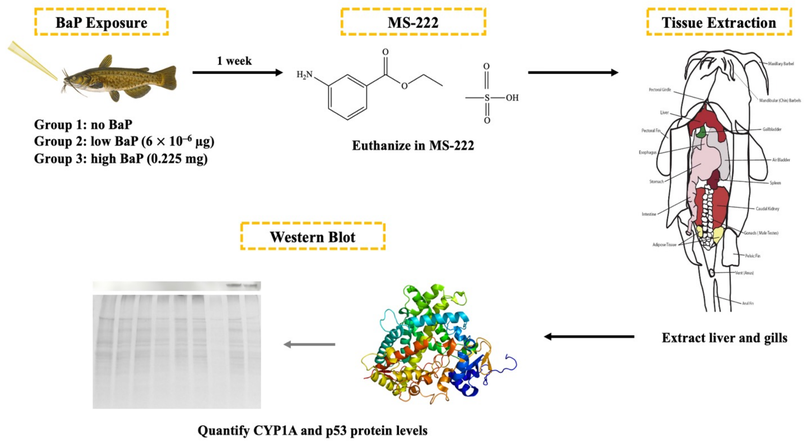

Figure 1. A stepwise representation of the experimental setup.

Fish were exposed to three different doses of BaP every other day for a week.

- Group 1 (control): pure olive oil with no BaP
- Group 2 (low-dose): 6 × 10^{-6} µg of BaP in olive oil
- Group 3 (high-dose): 0.225 mg of BaP in olive oil

After one week, fish were euthanized in tricaine methane sulfonate (MS-222), and survival time in MS-222 was measured. Liver and gill tissues were extracted for analysis of CYP1A and p53 protein levels with western blot.

Results 2

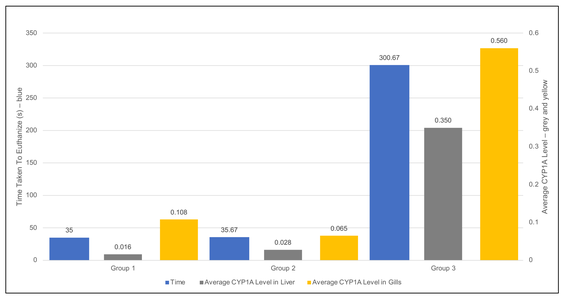

Figure 4. High levels of CYP1A correlates to longer survival time in MS-222. A correlation between average survival time taken in MS-222 and average CYP1A protein level in the liver and gills. The blue-colored bar represents the average time taken to euthanize; the grey-colored bar represents the average CYP1A level in the liver, and the yellow-colored bar represents the average CYP1A level in the gills.

- Group 3 under exposure to a high dose of BaP survived significantly longer in MS-222 when compared to the control group and group 2 (low dose of BaP).
- Group 3 also expresses higher levels of CYP1A protein in both liver and gills than group 1 and group 2.
  → Higher levels of CYP1A protein may lead to longer survival time in MS-222.

References

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Results 1

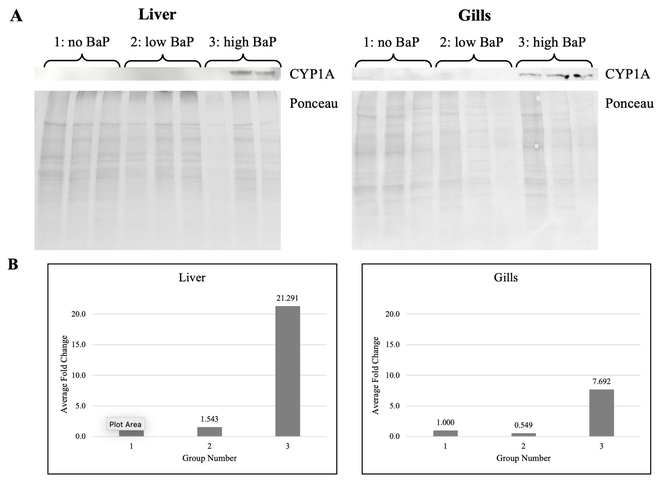

Figure 2. A high BaP dose leading to CYP1A induction in *Ameiurus nebulosus* liver and gills. (A) Visualization of p53 protein levels through chemiluminescence western blot detection then ponceau staining. (B) Quantitative analysis that compares the average fold changes of p53 levels with control. Fold change of group 1 (control) set to 1.

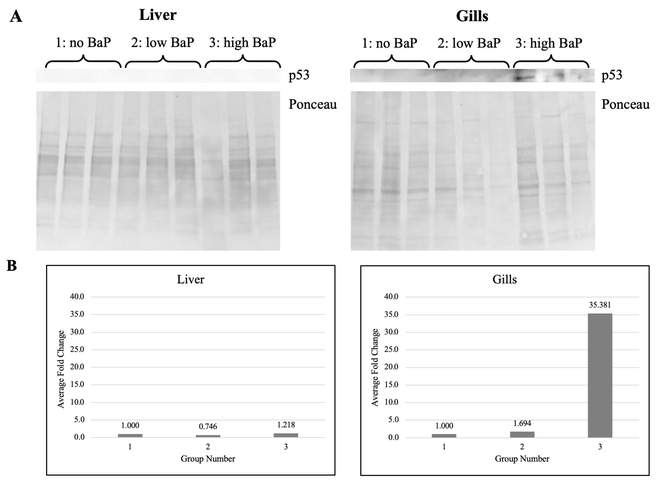

Figure 3. A high BaP dose leading to p53 induction in *Ameiurus nebulosus* gills. (A) Visualization of p53 protein levels through chemiluminescence western blot detection then ponceau staining. (B) Quantitative analysis that compares the average fold changes of p53 levels with control. Fold change of group 1 (control) set to 1.

- CYP1A protein levels increased significantly in group 3 under exposure to a high dose of BaP when compared to the control group and group 2 (low dose of BaP) (Fig. 2A).
- The fold change of the CYP1A levels in group 3 increased to 21.291 and 7.692 in the liver and gills, respectively, which are more than the standard value of the control (Fig. 2B).
  → A high dose of BaP leads to the induction of CYP1A proteins in *Ameiurus nebulosus*.
- p53 protein levels increased significantly in group 3 of gills under exposure to a high dose of BaP when compared to the control group and group 2 (Fig. 3A).
- The fold change of the p53 level in group 3 of gills increased to 35.381, which is more than the standard value 1 of the control (Fig. 3B).
- The liver did not show significant differences in p53 levels between the 3 groups.
  → A high dose of BaP leads to the induction of p53 proteins in the gills of *Ameiurus nebulosus*.