Effects of non-steroidal anti-inflammatory drugs on hormone production and gene expression in hypothalamic-pituitary-gonadal axis of zebrafish

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have shown estrogenic activity in vitro and in vivo, however, the mechanism of this activity is not fully understood.

We investigated the effects of NSAIDs on the hypothalamic-pituitary-gonadal (HPG) axis and related mechanisms in freshwater fish.

Materials & Methods

Test compounds:
- Five NSAIDs, i.e., acetylsalicylic acid (ASA), diclofenac (DCF), ibuprofen (IBP), mefenamic acid (MFA), and naproxen (NPX) were selected based on the frequency of detection in Korea waterways and their toxicity.

Experiment 1: fish exposure
- Four male and four female adult fish per group were exposed to 0, 10, 100, and 1,000 μg/L of five NSAIDs for 14 d with static-renewal method.
- Concentration of sex hormone in plasma and transcriptions of several genes along the HPG axis (brain and gonad tissue) were measured.

Experiment 2: fish exposure
- One most potent NSAID identified among the five NSAIDs was chosen and its effects on reproduction and development of the next generation were evaluated.
- Four male and six female fish were placed in a spawning aquarium, and exposed to 0, 0.1, 1, or 10 μg/L of IBP for 21 d following OECD TG 229.
- On 18th day, thirty eggs collected from each tank were placed in 48-well plate containing exposure water or clean water for 6 d.

F0 fish endpoints:
- Mortality, Egg production, Somatic index
- Sex hormone concentration, mRNA expressions of genes along the HPG axis

F1 fish endpoints:
- Hatchability, Time to hatch, malformation

Results & Discussion

Experiment 1: Effects of five NSAIDs on hormone and gene transcription
- Concentrations of 17β-estradiol (E2) in plasma were significantly increased in both male and female fish after exposure to IBP and MFA.
- Concentrations of plasma testosterone (T) were significantly greater in female fish, while T was decreased among the males.
- E2/T ratio was significantly greater in females exposed to ASA, IBP, MFA, and NPX, while more pronounced increase was observed in male fish exposed to IBP and MFA.

Effects of NSAIDs exposure on gene transcription were sex-dependent.
- Transcripts of β-estradiol receptor (ER), FSHR, and LHβ genes in males increased after the exposure to NSAIDs which could be potentially a cause for sex differentiation and malformation of the offspring.
- Test NSAIDs induced down-regulation of FSHβ, LHβ, and LHβ genes in males, suggesting possible delay in spermogenesis as well as male development.
- Possible explanation for the lower concentrations of plasma T in male zebrafish.

Continuous exposure to IBP through F1 generation increased malformation rates.
- Average number of eggs spawned was significantly less at 21 μg/L of IBP.
- Continuous exposure to 10 μg/L of IBP significantly reduced the rate of hatching.
- Parental exposure to 10 μg/L of IBP resulted in significant delay in hatching, even when they were transferred to clean water.

Continuous exposure to IBP through F3 generation increased malformation rates.
- These data indicate that parental exposure to low concentrations of IBP could influence the F3 generation, resulting in increased adverse effects in the offspring.

Concentration (μg/L)

Exposure to IBP affected transcription of genes of the HPG axis.
- Significant up-regulation of CYP17 along with greater plasma concentrations of E2 does not correspond with the suppressory properties of IBP.
- A possible explanation is that down-regulation of Est2, Est3, and Est4 mRNA in males might be compensation to greater production of E2 as a negative feedback.
- Changes in gene transcriptions were observed at 0.1 μg/L of IBP, while the environmentally relevant range.

Conclusion

IBP could modulate hormone production and related gene transcription of the HPG axis in a sex-dependent way, which could cause adverse effects on reproduction and the development of offspring.
- Potential ecosystem consequences of endocrine disruption by NSAIDs need further investigation.

For questions or comments please email me at jkh526@gmail.com