Naproxen (NPX) is a non-steroidal anti-inflammatory drug (NSAID) commonly used to alleviate pain and inflammation via inhibition of the enzyme cyclooxygenase (COX). In utero exposures to NSAIDs have been linked to preterm birth, neural tube closure defects, and orofacial malformations, which may be linked to abnormal neural crest cell (NCC) development. NCCs are stem-like cells that differentiate into numerous adult tissues including craniofacial cartilage and bone and neurons of the peripheral nervous system. Preliminary experiments in the Rogers Lab indicated that targeted knockdown of COX2 and its receptor, EP3, using translation-blocking morpholinos leads to aberrant NCC development in vertebrate embryos.

Our overall goal is to investigate the molecular mechanisms underlying the development of craniofacial defects following exposure to NSAIDs. We hypothesized that exposure to NPX during early development will inhibit cranial chondrogenesis. To test this hypothesis, we exposed Ambystoma mexicanum (axolotl) embryos to various concentrations of NPX during NCC migration and differentiation stages and then performed immunohistochemistry (IHC) for markers of NCC-derived cells. We identified that:

- NPX-exposed embryos show decreased survival and exhibit molecular changes at st. 28 and gross anatomic changes by st. 36.
- Exposed embryos have abnormal expression of SOX9 in NCCs. SOX9 loss results in abnormal spatial expression of Col2a and absent formation of discrete craniofacial cartilage structures.
- NPX also appears to disrupt normal RUNX2 expression and patterning in putative precursor cells of the lateral line sensory system.

We identified that the degree of exposure to NPX in axolotl embryos correlates with their rate of survival during development. There appears to be a significant decrease in survival rate between 5 and 7 days post exposure to NPX which corresponds with developmental stages 34-40 and progression of organogenesis.

**METHODS**

Axolotl embryos were divided into 4 treatment groups with corresponding concentrations of NPX in Holtfreter’s (HF) control. They were grown to various stages (st. 28, 36, and 45) and collected for IHC using one of the three sets of markers shown.

**RESULTS**

Survival during chronic naproxen exposure: NPX-exposed embryos exhibit decreased survival. The markers used in this project can be divided into 3 main groups: neural crest-specific markers (SOX9, PAX7, and SOX10), markers of NCC-derived chondrogenesis (SOX9, Col2a, and RUNX2), and non-chondrogenic NCC-derivative markers (TUBB3, GFAP, and Olig2).

**REFERENCES**


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