Effects of Valproic Acid on Somite Development in *Danio rerio*

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Objective

The purpose of this study is to examine the effects of valproic acid exposure on somite patterning and cartilage formation in Zebrafish embryos. Valproic acid affects the expression of *pax-1*, a gene product that regulates the differentiation of bone precursors in somites. This suggests the potential for valproic acid to disrupt early somite organization as well as early osteogenesis. Embryos will be treated with 0.1 M and 0.05 M valproic acid and scored for visible malformations.

Introduction

Valproic acid was first used as an anticonvulsant drug to treat epilepsy in the late 1960s. It has been shown to be a means of controlling seizures. However valproic acid is able to cross the placenta and has been linked to human birth defects (Polifka and Friedman, 2002), including facial and skeletal malformations. Other vertebrates show similar birth defects after exposure. For example, rats treated with valproic acid produced embryos with fused ribs, missing vertebrae, and kinky tails (Voorhees, 1987).

The teratogenic activity of valproic acid is thought to stem from its down-regulation of the *pax-1* gene thereby leading to skeletal malformations (Lammer et al., 1987). Pax-1 is a transcription factor that functions in the development of chondrocytes from the scelerotome portion of somites (Gilbert, 2003). These chondrocytes later give rise to portions of the axial skeleton, including vertebrae and portions of the ribs (Smith and Tuan, 1995). *Pax-1* expression is induced by Sonic Hedgehog protein from the notochord and is expressed in the ventral portions, or sclerotomes, of the somites. Specifically, Pax-1 protein is thought to be a necessary ventral fate patterning molecule required for the proper formation of the vertebrae (Koseki et al., 1993). *Pax-1* null mutant mice have little to no formation of the ventral portions of their vertebrae and vertebral discs (Wallin et al., 1994). Similar malformations are induced by treatment with valproic acid. Chicken embryos exposed to valproic acid also showed significant somite disorganization and malformations accompanied by a decreased level of *pax-1* expression (Barnes et al., 1996).

Materials Required

Mature zebrafish on 10 hr/14 hr-dark/light cycle
Valproic acid (Sigma P-4543)
Zebrafish embryo medium
60 mm glass Petri dishes (soap-free)
Wide-mouth glass Pasteur pipets (Fisher 13-678-30)
Incubator @28 degrees celsius

Procedure

1. Induce adult zebrafish to mate at the beginning of their light cycle by covering the bottom of the aquarium with a layer of marbles the night before. This will both induce the fish to mate and protect the embryos from predation. Leave overnight.

2. Harvest the embryos between one and three hours after the fish have awakened (at the appearance of light) by using a suction vacuum to clean the bottom, removing embryos from under the marbles into a filter.

3. Wash embryos from filter into a dish of aquarium water. Sort the embryos from extraneous debris by using a wide-mouth pipet to move them into a Petri dish of Zebrafish Embryo Medium (ZFEM) solution.

4. Stage the embryos and allow to develop to the time of first somitogenesis (approximately eleven hours after fertilization).

5. Prepare solutions of 0.05M valproic acid (VA) in ZFEM and 0.1M VA in ZFEM. Use ZFEM without VA as a control. Pour solutions into six separately labeled dishes.

6. Transfer between ten and twenty embryos to each of the six dishes. Incubate embryos at 28°C overnight. Observe and note malformations.

References

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