The effects of different concentrations of lithium chloride on the development of zebrafish embryos

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Objective

The purpose of this experiment is to monitor the effects of different concentrations of LiCl on anterior development in zebrafish embryos. This will be carried out by assessing the degree to which certain amounts of the lithium cholride teratogen affect the morphology of anterior structures such as the eye.

Introduction

Lithium chloride is a known teratogen which alters development in a variety of organisms including sea urchins, *Xenopus laevis* (frogs), and *Brachydanio rerio* (Zebrafish) (Gilbert, 2010). In sea urchin embryos, lithium chloride causes the accumulation of nuclear Beta-catenin in every cell, and transforms presumptive ectoderm into endoderm (Gilbert, 2010). Lithium exposure in cleavage-stage embryos of *Xenopus* inhibits dorsal/ventral axis specification and results in radially-symmetric, dorsal-anteriorized embryos (Stachel et. al., 2003). The research conducted by Stachel and colleagues suggests that lithium induction of pre-midblastular Zebrafish prevents normal dorsal/ventral axis patterning by acting as an inhibitor to the phosphoinositol pathway, which results in *goosecoid* and *noggin* expression outside the region of the presumptive embryonic shield instead of these genes being confined to the region proximal to the dorsal blastopore lip.

Experiments by Stachel and colleagues have shown that the development of anterior structures is dependent on Wnt signaling, especially the transcription of *goosecoid*, which codes for a dorsalizing protein necessary for normal anterior development (Stachel et. al., 2003). The increase in gene expression of organizer-specific proteins such as Goosecoid in the presumptive ventral regions of the organism produces different phenotypic results when the induction occurs at certain stages in development (Stachel et. al., 1993). For instance, the exposure of embryos to LiCl before the midblastular transition (2 hour stage) results in hyperdorsalization and the inhibition of normal dorsal/ventral axis patterning (Deitrich, 1999). In contrast, embryos exposed to LiCl at the four-hour stage after the midblastular transition experienced normal dorsal/ventral axis specification but perturbed development of anterior structures such as eyes (Stachel et. al., 1993).

Materials

- 60-80 *Brachydanio rerio* embryos at the sphere/dome-stage of development
- Zebrafish embryo medium.
- LiCl (0.15 M, 0.30 M, and 0.45 M) in Zebrafish embryo medium
- Nine 60 mm glass petri dishes
- wide-mouth pastuer pipette
- dissecting microscope

Procedure

1. Obtain 15-20 zebrafish embryos at the sphere/dome-stage of development (four hours post-fertilization) for each of the four titrations of lithium chloride to be tested, inclusive of the control group. Therefore, about 60-80 sphere/dome-stage embryos must be isolated in total.

2. Place the embryos into four separate petri dishes, each containing 10 mL of Zebrafish embryo medium or the following concentrations of LiCl:
    - 0.45 M
    - 0.30 M
    - 0.15 M

3. Transfer each population of embryos from the embryo medium to the LiCl-containing dishes using a wide-mouth pipette, and immerse them in solution for 10 minutes.

4. After 10 minutes, rinse the embryos by placing each group in a separate dish containing 10 milliliters of embryo medium, and then transfer to fresh embryo medium. Be sure to label the dishes with the corresponding amount of LiCl to which they were induced.

5. Photograph the embryos after 24 hours to observe anterior development, carefully noting deviations in anterior patterning and formation from the control population.

References

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Fish/lithium.html

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Table I: Variable Molarity of Lithium Chloride Titrations

Formula weight	Experimental concentration (M)	Conversion factor	Amount LiCl added
42.39 g/ 1 mole	0.45 M/ L	.01 L/ 10 mL	0.19 g/ 10 mL
42.39 g/ 1 mole	0.30 M/ L	.01 L/ 10 mL	0.13 g/10 mL
42.39 g/ 1 mole	0.15 M/ L	.01 L/ 10 mL	0.06 g/ 10 mL