Using a Sensitive Japanese Medaka (Oryzias latipes) Fish Model for Endocrine Disruptors Screening

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research & development

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Science Question

The endocrine system consists of a highly integrated set of glands and widely distributed neuroendocrine cells in exocrine glands whose primary function is the control of homeostasis, including growth, reproduction and fertility, which are essential for propagation of the species. Therefore, the disruption of the endocrine system by any anthropogenic chemical may eventually have profound effects on both individuals and the population of a species. Endocrine disrupting chemicals (EDC's) are under intense scientific scrutiny because of the increasing number of environmental contaminants linked to disruption of one or more components of the endocrine system. Perhaps the greatest challenge to scientists studying EDCs are that (1) many of these chemicals can exert their effects at very low doses, and (2) harmful effects may not manifest for months or years after exposure. Thus, it is possible that many of the pesticides and industrial products in widespread use today may have harmful long-term effects that interfere with some aspect of the endocrine system. Although there is no single approach that can simultaneously identify EDCs and characterize their toxicity, the development of a rapid, sensitive, biologically-integrated screening assay that can identify and classify EDCs by category of endocrine activity is of utmost importance in beginning to understand the scope of the problem posed by these chemicals.

Research Goals

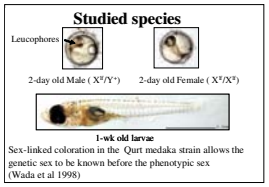
Our goals are to develop and validate a high-throughput EDC screening assay utilizing a microarray gene chip. Four objectives are proposed:

1. Develop a microarray chip designed to detect endocrine disrupting chemicals,
2. Identify gene expression profiles associated with six categories of endocrine disrupting activity:
3. Conduct a statistical analysis of gene expression profiles to develop response criteria that identify patterns predictive of endocrine disruption; and
4. Validate the microarray chip using a set of chemicals selected to represent both positive and negative controls, as well as chemicals with previously-undefined endocrine activity.

Six Prototypical EDCs

1. 17-β Estradiol (E2) is one of the most studied natural estrogens
2. ICI 182,780 (Faslodex™, AstraZeneca, UK) is a novel, steroidal estrogen antagonist that was specifically designed to be devoid of estrogen agonist activity (Howell et al., 2000).
3. 11-Ketotestosterone is the primary endogenous fish androgen and a non-aromatizable androgen (Borg, 1994). Since it is not converted to estradiol by P450 aromatase, the biological effects observed in exposed Qurt medaka fish should be those expected from a pure androgenic agent
4. Flutamide is a non-steroidal compound widely used in endocrinology research. It has been thoroughly established that this therapeutic agent can function as an antiandrogen in mammals (Bayley et al., 2002)
5. 3, 3', 5-Triiodo-L-thyronine (T3) is one of two iodinated hormones secreted by the thyroid gland. T3 is essential for growth, differentiation, and reproductive system development (Dace et al., 2000; Choksi et al., 2003). T3 primarily exerts its biological effects through a receptor-mediated mechanism. Furthermore, T3 modulates its own function by regulating the thyroid receptor (TR) levels (Dace et al., 2000)
6. Amiodarone is a diiodinated benzofuran derivative that functions as a thyroid receptor antagonist (Shahrara and Drvota, 1999; Latham et al., 1987)

Studied species



Sex-linked coloration in the Qurt medaka strain allows the genetic sex to be known before the phenotypic sex (Wada et al 1998)

Methods/Approach

Experimental Design I

Determining biologically effective concentrations (BECs) of the six prototypical EDCs

Type of experiment: 96-h static renewal method
Sample size: 50 larvae per gender per four replicates
Time of Sampling: Three months after exposure
Endpoints:
1. Health indices (body length, body weight, and condition factor)
2. Histopathology
3. Occurrence of intersex

Experimental Design II

Developing Custom Medaka 12K Oligonucleotide Array

Features of array:
1. EDC-affected genes (n =131)
2. Medaka cDNAs (n = 1075)
3. Medaka genome project (n = 890)
4. Unigene database (n = 8154)

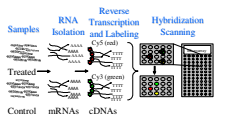
9379 unique and ~3000 control probes

Experimental Design III

Identifying unique gene response patterns of the six prototypic EDCs

Type of experiment: Static 6-h exposure to
1 Ethanol control
2 10 μg/L 17-β Estradiol
3 10 μg/L ICI 182,780 (Faslodex™)
4 34 μg/L Amiodarone
5 100 μg/L 11-Ketotestosterone
6 500 μg/L 3, 3', 5-Triiodo-L-thyronine
7 1000 μg/L Flutamide

Sample size = 200 larvae per gender per three replicates
Total RNA isolation
Microarray analysis
Analysis of microarray images
Loess normalization and functional embedding for high dimension data analyses
Latent ordering of genes for pattern analysis and identification



Results

Testicular Histopathology

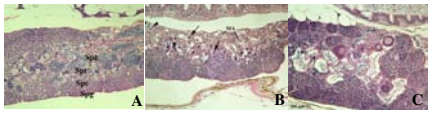


Figure 1. (A) Normal testis of control male showing several reproductive stages in spermatogenesis (Spg = spermatogonia; Spc = spermatocytes; Spt = spermatids; Spz = spermatozoa), (B) Ovatestis in a male exposed to 320 μg/L flutamide. Notes: Both severe germ cell necrosis (arrowheads) and ova cell necrosis (arrows) were observed in this intersex fish (MA = macrophage aggregations), and (C) Ovatestis in a male exposed to 500 μg/L of 3, 3', 5-Triiodo-L-thyronine.

Thyroid Histopathology

Figure 2. (A) Normal thyroid gland in control male showing single-layer of low cuboidal follicular cells, (B) Follicular cell atrophy (arrow) in male exposed to 50 μg/L of 3, 3', 5-Triiodo-L-thyronine, and (C) Thyroid follicular cells hyperplasia and hypertrophy were observed in males exposed to 500 μg/L of 3, 3', 5-Triiodo-L-thyronine. Arrows point to necrotic follicular cells.

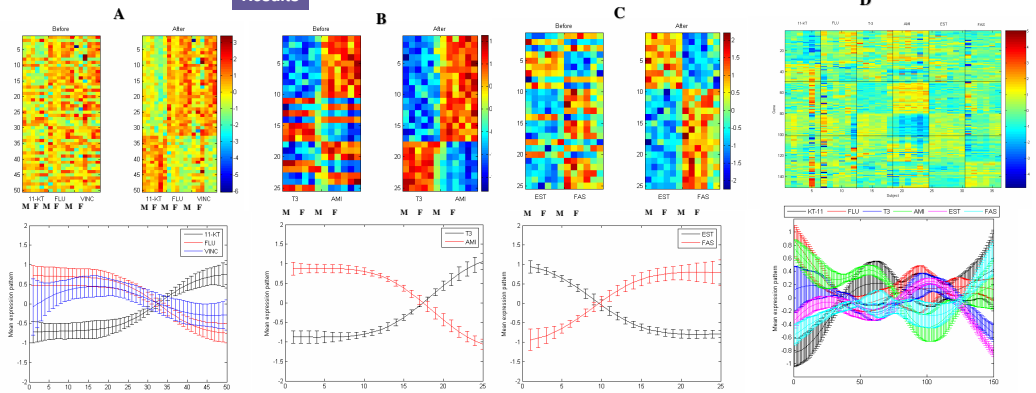


Figure 3 A-D. Heat map images of the six prototypical EDCs before and after application of a functional embedding technique (Muller and Wu, 2006). To perform the clustering/classification analysis, the latent ordering of genes with the smallest p-values (based on a t-test) was used to express the subject profiles (columns) in functional form. The heat map images were then transformed to line curves using the scattered smoothed mean curves with standard error bars. 11-Ketotestosterone = 11-KT, Flutamide = FLU, Vinclozolin VIN, 3, 3', 5-Triiodo-L-thyronine =T3, Amiodarone = AMI, 17-β Estradiol = EST, and ICI 182,780 (Faslodex™) = FAS.

Discussions/Conclusions

1. We developed a custom medaka microarray chip, and tested its ability to discriminate between 6 prototypical EDCs evaluated at BECs.
2. The medaka microarray, coupled with robust statistical techniques, can differentiate between 6 biological categories of EDC on the basis of gene expression patterns (Fig 3).
3. The expression profile for vinclozolin is remarkably similar to the prototypical antiandrogen flutamide, indicating that our system can prospectively identify EDCs with characteristic modes of action.
4. Tests of the microarray are in progress with additional EDCs and simple EDC mixtures.

Impact and Outcomes

The medaka microarray chip will provide a powerful screening tool that can simultaneously yield information on not only the parent chemical's ED activity but also any active metabolites. The basic screening system is also directly amenable to the future characterization of organ-specific responses to EDCs, as well as to characterizing differences in EDC gene-expression profiles associated with exposure of different life stages. Furthermore, assessment of complex contaminant mixtures of EDCs will be possible with this novel method and could greatly facilitate future aquatic environmental EDC identification and evaluation.

Future Directions

Our goal is to make our system into a comprehensive tool for screening and identifying previously-uncharacterized EDCs. We will seek funding to perform (1) time-course and (2) dose-response experiments with the prototypical EDCs; (3) develop integrative software to facilitate data analysis; and (4) screen numerous additional chemicals - both individual compounds and mixtures.

With medaka genome annotation, we will identify the function of affected genes and gene pathways, laying the groundwork for cross species comparisons on shared mechanisms and vulnerabilities to these compounds.

References

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Long Term Goal II