Developmental Exposure to Endocrine Disruptors: Fertility and Gene Expression Profiles

Project Scope

Selected epidemiological studies have reported dramatic decreases in sperm counts over the last 50 years, and results from previous rodent studies indicate that potent estrogenic chemicals, such as diethylstilbestrol (DES) can adversely affect sperm counts and quality. Epidemiological and experimental evidence had also prompted a concern that exposure to weakly estrogenic substances during critical periods of development may result in compromised fertility in sexually mature adults. Such exposures may contribute decreased sperm counts and sperm quality in sexually mature offspring.

The presence of estrogenic compounds such as genistein, found in soy products, has been associated with several health benefits when consumed by human adults, but early developmental exposure to genistein has been shown to have negative effects on reproductive function. Although genistein and related estrogenic chemicals do not share obvious structural similarity to estrogen, it is believed that their effects are nonetheless mediated by the interactions with estrogen receptors, and result from the inappropriate modulation of gene expression at critical times during development. Other mechanisms of action that are independent of the estrogen receptor cannot be discounted, however.

The long-term goal of this study was to determine the effect of developmental (i.e., gestational and lactational) exposure to estrogenic chemicals on sperm counts, sperm quality, and reproductive fitness in adult male mice, and to associate these effects with changes in gene expression profiles in the testis, epididymis, and sperm.

The main objectives of this research were to:

- Examine the physiological (i.e. sperm counts and quality) and molecular (i.e. testicular and epididymal gene expression profiles) effects of developmental exposure to DES, a known strongly estrogenic compound;

Grant Title and Principal Investigator

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Key Findings and Implications

- Prenatal and lactational exposure to diethylstilbestrol (DES) causes long-term adverse effects on testicular development and sperm function in mice, and these effects are associated with changes in testicular gene expression, even long after the cessation of DES exposure.
- Developmental exposure of male mice to genistein at dietary levels equal to or exceeding that of human populations consuming a soy-rich diet does not result in adverse effects on sperm quality or changes in testicular gene expression similar to DES.
- *In utero* and lactational exposure to genistein at levels as high, or higher, than human populations consuming a soy-rich diet does not affect the morphology of the mammary gland in pubertal female mice.
- Data demonstrate the potential for developmental exposure to DES, and possibly other estrogenic chemicals, to irreversibly alter testicular growth, sperm function, and testicular gene expression, including genes involved in steroidogenesis, lysosomal function, and testicular development.
- These findings suggest that estrogenic endocrine disruptors may act through multiple mechanisms of action, and estrogenic potency alone may not be an accurate predictor of adverse reproductive effects.

Publications include eight peer reviewed journal articles.

Project Period: September 1999 to July 2002

- Investigate the effects of genistein (GEN) and ethynyl estradiol (EE), two environmentally relevant but weak estrogenic endocrine disruptors, on the same endpoints.

Male mice were exposed to DES, GEN, and EE during gestation (orally to the dam) and via lactation until weaning at postnatal day 21. Sperm quality was assessed in the exposed male offspring at 15 and 45 weeks using sperm motion analysis and in vitro fertilization assays. Pups were monitored for several developmental landmarks (e.g., body weight, anogenital distance). Changes in gene expression in the testis and epididymis were examined in control and treated animals at 3, 15, and 45 weeks using a customized genome-scale microarray enriched for genes expressed in the mouse testis and epididymis. Microarray technology provides a high-capacity assay to simultaneously monitor the modulation of expression of hundreds of genes and to identify treatment-related, gene-expression changes.

General Linear Modeling (GLM) was used as a novel statistical approach to discern dose-dependent and temporal associations between effects on sperm quality and changes in gene expression profiles. Cluster analysis was used to sort genes into groups that responded in a similar fashion to each agent. Results from these statistical analyses were used to identify critical target genes, common effects on gene expression patterns, and to discern possible mechanisms involved in estrogen receptor-mediated modulation of sperm parameters. The gene expression data were used identify novel biomarkers of testicular/epididymal toxicity that correlated with adverse affects on sperm counts and quality.

Several computational resources were developed to assist with the analysis and storage of microarray data. This included an automated and customizable program to correct, filter, and normalize raw microarray data. Several new statistical methods were evaluated and compared to conventional approaches. Microarray analysis tools were subsequently used to identify early and latent alterations in the expression of genes involved in estrogen signaling, steroidogenesis, lysosomal function, and regulation of testicular development in DES-treated animals. Mixed models were fitted to the microarray data to determine precisely which genes were most likely driving the patterns observed in the preliminary analyses. The approach centered around two interconnected mixed models, a "normalization" model, which accounted for experiment-wide systematic effects that could bias inferences made on the data from individual genes, and a "gene" model, which was fitted separately to the normalized data for each gene, allowing for inferences to be made using separate estimates of variability.

The effects of DES and GEN on mammary gland development also were assessed in developmentally exposed offspring. Mammary gland whole mounts were examined on postnatal day 49 for epithelial growth (percent of fat pad occupied by epithelium), length of mammary epithelial tree from nipple to distal edge, number of terminal end buds (undifferentiated proliferating structures), and density of alveolar buds (differentiated milk secreting lobules). In addition, the effect of gestational and lactational exposure to DES, GEN, and EE on ovulation and egg fertilizing ability in sexually mature female offspring were investigated.

Relevance to ORD's Multi-Year Research Plan

This project contributes to ORD's Multi-Year Plan long-term goal of providing a better understanding of the science of underlying effects of endocrine disruptors. For the purposes of risk assessment, revealing correlations between physiological effects and changes at the molecular level (i.e. modulation of gene expression profiles) is critically important in order to establish potential mechanisms of action and to confirm that changes in the expression of gene networks translate into the manifestation of a toxic response. Such "core" research contributes to the development of improved methods for risk assessment and risk management and benefits EPA's program and regional offices, other federal agencies, agencies in other countries, and the scientific community at large. Moreover, results from this project may identify novel gene targets that are involved in the toxicity of endocrine disruptors, which could lead to the development of biomarkers for reproductive toxicants.

Project Results and Implications

Developmental exposure to DES, GEN, and EE affected sperm quality and sperm counts, but in different ways. For example, DES compromised fertility by decreasing sperm counts and quality. In contrast, GEN significantly increased fertility in the high-dose group with no significant treatment-related effects on body weight, anogenital distance, seminal vesicle weight, or testis weight. The doses used in the study were considerably higher than the estimated human intake. EE decreased sperm concentrations and the number of motile sperm, but there was no treatment effect on in vitro fertilizing ability.

The microarray data analysis confirmed that DES markedly affected patterns in testicular and epididymal gene expression, and that the effects vary strongly across developmental stages. PCR analyses revealed that early exposure to GEN at the doses tested had little or no effects on the expression pattern of these same genes. These results suggest that the GEN does not act as an estrogen agonist under conditions of the experiment.

The effect of early GEN on female reproduction has not previously been described. Neither DES nor GEN exposure affected anogenital distance in the offspring of exposed dams. A positive, but not significant, relationship was observed between DES exposures and mammary gland size, while early exposure to GEN did not affect mammary growth, or the number of terminal or alveolar buds in pups from exposed dams. Ongoing research is following up on preliminary data suggesting that early exposures to DES, GEN and EE affect ovulatory cycles in exposed offspring.

Investigators

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For More Information

Zacharewski Lab Home Page and dbZACH Toxicogenomic Database:
http://dbzach.fst.msu.edu/
http://www.bch.msu.edu/~zacharet/

NCER Project Abstract and Reports:
http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/451/report/0