CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Samiha Mahin

Project Number
38675

Project Title
In Vitro Effects of Genistein and Di-(2-ethylhexyl) Phthalate on Testicular Macrophages and Spermatogonial Cells

Abstract

Objectives/Goals
(1)Determine whether Gen and MEHP, alone or mixed, directly target macrophages and affect their pro-inflammatory responses in-vitro. (2)Determine whether Gen and MEHP, alone or mixed, directly target spermatogonia stem cells, precursors of spermatozoa.(3)Determine whether Gen and MEHP change the interactions between macrophages and spermatogonia.

Methods/Materials
MHS Cell line cultured as a model for testicular macrophages and C18-4 Cell line cultured for spermatogonial cells.
(1)MHS was stimulated with Lipopolysaccharide (LPS) to induce the inflammatory response of macrophages. After treating and culturing the cells, MHS had gone through RNA extraction, cDNA synthesis and Quantitative Real-Time PCR (qPCR). (2)The spermatogonial cells were treated for 24 hr and 48 hr with Gen and MEHP and then analyzed with MTT assay. (3)For co-culture treatments, RAW 264.7 macrophage cell line was used as a model for testicular macrophages. RAW grew in transwell inserts, pre-treated with Gen and/or MEHP for 21 hrs. C18-4 viability was then analyzed with MTT assay.

Results
(1)Gen and MEHP alter the basal and LPS induced production of inflammatory cytokines by macrophages. The mRNA relative expression showed that macrophage activity can be altered by Gen and MEHP, which could have consequences for testis functions in vivo. (2)Gen and MEHP combination at the highest concentration increased viability/proliferation of spermatogonial cells at both 24 hr and 48 hr treatments. So, this confirmed my hypothesis that these EDCs can directly target spermatogonia, which may affect spermatogenesis. (3)The presence of macrophages pre-treated with Gen and MEHP, alone or in combination, and activated or not by LPS did not have an effect on the viability/proliferation of spermatogonial cells after 48-hour co-culture.

Conclusions/Discussion
Changes in the mRNA relative expression of cytokines confirmed the hypothesis that Gen and MEHP can alter directly macrophages and change their biological responses. MTT assays suggest that Gen-MEHP mixture affect spermatogonial viability or proliferation, while Gen and MEHP had no effect alone. For both cell lines, MEHP and Geni did not behave the same way when together or alone. These studies will help understand how the effects of the two EDCs early in life on macrophage and spermatogonia may play a role in their long-term negative effects on male reproduction.

Summary Statement
I demonstrated that the endocrine disruptors, Genistein and DEHP, can directly alter testicular function and maintenance such as a pro-inflammatory in macrophages and proliferation in spermatogonia.

Help Received
I have conducted this research at the USC School of Pharmacy under the supervision and mentorship of my Principal Investigator, Dr. Martine Culty and the assistance of the postdoctoral student, Dr. Vanessa Brouard.