assessed for changes in histomorphology, hormone receptor expression, immune cell number, and gene expression. We found that PP reduced many of the typical morphological effects of parity on the mammary gland, resulting in intermediate phenotypes for ductal density and total epithelial structures. Notably, we found increased proliferation in PP-treated mammary glands, despite decreased ductal epithelial volume relative to parous controls. Mammary glands from PP-treated females also had alterations in the expression of ERα-mediated genes, including PgR (the gene that encodes progesterone receptor) and Igf1, with expression levels that were intermediate to both nulliparous and parous control mice. Finally, PP reduced the effect of parity on several immune cell types in the mammary gland including B cells, T-cells, and M2 macrophages. These results suggest that PP, at levels relevant to human exposure, can disrupt the normal response to parity in the mouse mammary gland, including persistent alterations to mammary gland structures. Future studies should address whether PP exposures disturb the protective effects of pregnancy on mammary cancer risk.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Expression Patterns of Analgesic Metabolising Machinery in 1st and 2nd Trimester Human Fetal Liver and Gonads

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Use of over-the-counter analgesics during pregnancy is widespread globally. Most analgesic compounds can freely diffuse through the placental feto-maternal interface and reach the developing fetus. Current literature suggests an endocrine disruptor (ED) potential of *in utero* exposure to these compounds. The liver is the primary site of contact with EDs in the fetus. Exposure of the fetal gonads can also alter reproductive function with potential intergenerational effects. We aimed to characterise the metabolic capability of these fetal organs. RNA sequencing was performed in 80 second trimester human fetal livers and 48 fetal gonads (balanced for fetal age and fetal sex). Samples were collected from elective terminations of normal pregnancies (liver 11-19 weeks, FeGo study: REC 04/S0802/21, and gonads 6-17 weeks, as previously described[1]. RNA was extracted and Illumina NextSeq was used to produce 76 bp single end (liver) or paired end 2x50 bp (gonads) sequencing reads. Reads were quality controlled, aligned to the human reference genome and quantified at gene regions. Statistical analyses involved an ANOVA model of two-way interactions between fetal sex and fetal age. All organs expressed phase I and II metabolising enzymes and drug transporters involved in the pharmacokinetic and pharmacodynamic pathways of over-the-counter analgesics. The human fetal liver expressed ABCC2, ABCC3, ABCC4 and ABCG2 receptors at similar levels between males and females. Expression of cytochrome p450 enzymes CYP2A6, CYP2C8, CYP2C9, CYP2E1, CYP3A4 involved in metabolism of the analgesics paracetamol and ibuprofen, all increased with gestational age in the liver. Expression of GSTM1, GSTP1, GSTT1, SULT1A1, SULT1A3, SULT1A4, SULT1E1, SULT2A1, UGT2B4, UGT2B7 and UGT2B15 metabolising enzymes also increased during gestation, while fetal hepatic GSTP1 expression showed a significant 2-way interaction between both sex and age. Fetal gonads expressed ABCC4 and ABCG2 transporters, with transcript levels demonstrating significant sex-specific and gestational age differences. Fewer analgesic metabolising enzymes were expressed in the gonads than the fetal liver, including CYP2E1, GSTP1 and SULT1A1, all significantly altered by gestation and fetal sex. Our results reveal expression of major analgesics metabolic and transport components within the human fetal liver, ovaries and testes between gestation weeks 7-19. Significant sex alterations in transcript levels also suggest sexually dimorphic metabolic activity of these organs during fetal life. In conclusion, analgesics can be transported into fetal liver and gonad cells and metabolised into bioactive forms, posing toxicity risks for the developing fetus.1. Lecluze E, Rolland AD, Filis P, et al. Dynamics of the transcriptional landscape during human fetal testis and ovary development. *Hum Reprod*. 2020;35(5):1099-1119.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Hyperthermia as a Steroidogenic Inhibitor of Adrenodortical Cells, an Adrenal Sparing Treatment for Primary Aldosteronism?

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Introduction: Primary Aldosteronism (PA) is the commonest secondary cause of hypertension. Mainstay therapy, adrenalectomy resects both hypersecreting and adjacent normal tissue. It is therefore only suitable for patients with unilateral disease (40% cases), whom are surgical candidates. Thermal therapy presents a plausible minimally invasive therapeutic, to target and disrupt hypersecreting adrenal nodules in primary aldosteronism, while also preserving adjacent normal adrenal cortex.
Methodology: Adrenocortical cell lines (H295R and HAC15) were treated with hyperthermia using a water bath at temperatures between 37-65^{0}C for 15 minutes. Cell death and apoptosis were analysed immediately, 24 hours, and 48 hours post hyperthermia using Annexin V / Propidium Iodide (PI) (flow cytometry), and Calcein / PI imaging techniques. Steroidogenic potential was also analysed post hyperthermia by (i) measuring cytosolic calcium flux in response to angiotensin II (ANGII) using Flou-4 staining (flow cytometry); (ii) measurement of steroidogenic enzyme expression (RT-PCR); (iii) by measurement of cortisol