Guevara, personal communications). *T. cruzi* infection has been shown to directly modulate pituitary hormones such as GH, PRL and glucocorticoids (stress related hormones), leading to immunosuppression and thymic atrophy by depletion of $CD4^+$ $CD8^+$ cells. Previously, rats infected with *T. cruzi* and treated with GH showed reduced parasitism and less tissue damage compared to controls (2). The purpose of this research is to investigate the *in vitro* effect of GH during *T. cruzi* infection, simulating conditions of GH insensitivity. First, we separately treated *T. cruzi* and the host cells [human cervical cancer cell line (HeLa) and male mouse fibroblast (L-cells)] with relatively low or high levels of GH, IGF-1, PRL, and EGF. Next, we treated the parasite and host cells simultaneously with these hormones. When the parasites were treated alone, *T. cruzi* responded to exogenous GH (5ng/ml-50ng/ml) by significantly increasing the percentage of amastigotes (less infective form of the parasite). Also, when GH (50ng/ml) were administered to the host cells, *T. cruzi* infectivity was significantly reduced by 12% (percentage of infection) compared to 20% from untreated conditions. Similarly, both parasite and host cells treated with GH significantly reduced *T. cruzi* infectivity (10%) compared to untreated conditions (18%). We further treated both cell lines with a combination of GH/IGF-1. Conditions used were as follows: control (no-treatment), moderate levels (5ng/ml GH+150 ng/ml IGF-1), relatively high levels (50ng/ml GH+600ng/ml IGF-1), or levels that would simulate those found in patients with LS(50ng/ml GH+20 ng/ml IGF-1). Of these, the LS concentrations significantly reduced infection in both cell lines (11%) compared to control (16%). Together these results indicate that GH can influence *T. cruzi* infectivity and that GH, not IGF-1, is mediating the decreased infectivity. Finally, the results suggest that high concentrations of GH, as seen in LS patients, could be protective during *T. cruzi* infection.
1)Guevara-Aguirre et al., 2011 2) Frare et al., 2010

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

The Effects of Naphthenic Acids on Tryptophan Metabolism and Peripheral Serotonin Signalling

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Introduction: Serotonin produced in the periphery has been shown to affect glucose and lipid homeostasis. The availability of the amino acid tryptophan, the precursor of serotonin, affects serotonin availability. In addition, the metabolism of tryptophan via the kynurenine pathway produces physiologically active metabolites which have been shown to be altered under conditions of increased adiposity and dysglycemia. There is now evidence demonstrating some environmental xenobiotics, known to affect glucose and lipid homeostasis, can also alter serotonin production and key components of the kynurenine pathway. Recent evidence suggests that exposure to compounds present in petroleum and wastewaters from oil and gas extraction sites can impact endocrine signaling and result in aberrant lipid accumulation and altered glycemic control. However, whether any of these changes can be causally ascribed to altered serotonin synthesis/signaling or tryptophan metabolism remains unknown. The goal of this study was to determine the effects of exposure to naphthenic acid (NA), a key toxicant found in wastewater from bitumen (thick crude oil present in oil sands deposits) extraction on the enzymes involved in tryptophan metabolism and serotonin production.
Methods: McA-RH7777 rat hepatoma cells, were exposed to a technical NA mixture for 48 hours at concentrations within the reported range of NA found in wastewaters from oil extraction. We assessed mRNA expression for key rate-limiting enzymes involved in tryptophan metabolism that lead to either serotonin [*Tph1*] and/or kynurenine [*Ido2* and *Tdo2*] production, as well as downstream enzymes in the kynurenine pathway [*Afmid*, *Kyat1*, *Aadat*, *Kyat3*, *Kmo*, *Haao*, *Acmsd*, *Qprt*]. We also examined the effects of NA on prostaglandin synthesis [*Ptgs1*, *Ptgs2, Ptges*] and signalling [*Ptger2*, *Ptger4*] as prostaglandins have been shown to be induced by serotonin and are linked to hepatic fat accumulation.
Results: NA treatment significantly increased *Tph1* and *Ido2* expression; this occurred in association with a significant increase in the expression of the inducible prostaglandin synthase *Ptgs2* (COX-2), prostaglandin E synthase *Ptges,* and prostaglandin receptors *Ptger2* and *Ptger4*. *Acmsd* was the only downstream enzyme in the kynurenine pathway that was significantly altered by NA treatment.
Conclusion: These results provide proof-of-concept that compounds associated with oil sands extraction have the potential to perturb key components of serotonin synthesis (*Tph1*) and tryptophan metabolism (*Ido2*, *Acmsd*). Furthermore, we found that the increase in *Tph1* expression paralleled expression of *Ptgs2*. As increased prostaglandin production has been reported in association with nonalcoholic steatohepatitis, these data provide a potential mechanism by which exposure to NA and other petroleum-based compounds may increase the risk of metabolic disease.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

The Effects of Tert-butyl Hydroquinone (TBHQ) on Estrogen Receptor Alpha (ERα) and Tumor Suppressor Gene p53 in Breast Cancer Cells

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Tert-butyl hydroquinone (TBHQ) is an aromatic compound that is commonly used as a preservative in processed food to prevent rancidity and lengthen shelf life. TBHQ is known to act as an antioxidant by protecting cells from radical oxygen

species and thus preventing DNA damage. Although previous studies have found TBHQ to cause cancer cell death at high concentrations, they have also contrastingly found TBHQ, when studied in animal models, to enhance carcinogenic effects. However, the effect of TBHQ on breast cancer has not been thoroughly explored. With the prevalence of breast cancer and the wide use of TBHQ in processed food items, it is imperative that we explore their possible relationship. This study examined the effects of TBHQ, alone and in combination with hormones and anti-hormones, on ERα and p53 expression in both MCF-7 and T-47D breast cancer cell lines. To ensure treatment conditions without the presence of endogenous steroids or growth factors, the cells were cultured with a 5% charcoal-stripped fetal bovine serum (FBS) for six days. Western blot analysis revealed alterations in the expression of ERα and p53 protein levels after 24 hours of treatment with varying concentrations of TBHQ (0.005 to 1 mM). A concentration-dependent decrease of ERα protein levels was observed in both cell lines, with a 49% reduction occurring with 100 μM TBHQ as compared to the control. P53 levels portray a continued increase of expression through concentrations of TBHQ (0.005 to 1 mM), found similarly in both cell lines. To gain further insight into possible similarities between BPS and other known effectors of ERα, the optimal concentration of TBHQ (100 μM) was used in combination with hormones and anti-hormones. Down-regulation of ERα protein levels was observed after 24-hour co-treatment of T-47D & MCF-7 cells with a combination of TBHQ and E_2. Antiestrogen ICI with TBHQ showed a significant down-regulation as compared to TBHQ alone, and TBHQ with TAM portrayed no significant differences. A similar trend in the effects on p53 expression was depicted in T-47D and MCF-7 cells. Image cytometric analysis with propidium iodide staining was utilized to quantify cell values and viability changes to further portray the effects of TBHQ on T-47D and MCF-7 cellular growth. The viability assay shows a biphasic effect with increasing concentrations of TBHQ, with a maximum decrease in proliferation seen at a concentration of 100 uM TBHQ. TBHQ alone and in combination with E_2 and antiestrogens showed a decreased proliferation compared to the control in T-47D cells. However, cytolocalization of ERα upon treatment with estradiol and TBHQ remained unaltered. Our studies offer a unique perspective on the effects of TBHQ on two different breast cancer cell lines, and provide valuable insight for further exploration of the mechanism of action of TBHQ on tumor suppressor gene and steroid receptors.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Thyroid Gland and Male Reproductive Anomalies Among Fuel Handlers in Gampaha District, Sri Lanka

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Introduction: Fuel handlers at petrol stations are continuously exposed to organic solvents from fuel and vehicle emissions. Endocrine disrupting chemicals (EDC) are present in fuel, which are harmful to endocrine organs. Thyroiditis and hypogonadism are reported among fuel handlers. Thyroid gland and male reproductive function anomalies were investigated among fuel handlers in the Gampaha district of Sri Lanka. **Method:** 43 were recruited from 6 fuel stations in the Gampaha district for the study and 28 age matched male workers who were not exposed to fuel in an occupational setting were recruited as controls. Thyroid gland was examined clinically and TSH, free T4, FSH, LH and Testosterone were done on all the participants. TPO antibody and a thyroid scan was done on the fuel handlers. **Results:** Median (IQR) age was 38 years (27-46 years). The mean TSH value was 1.62 IU/mL (1.15-2.35) vs 1.33 IU/mL (0.83-1.79) respectively in study and control populations with significantly higher levels in the study population (p=0.023). The median (IQR) TSH value above the reference range was identified in 7% of fuel handlers and all controls were within the normal range, while 16.9% of fuel handlers had a derangement in the TPO levels. On examination, only one control had a small goiter but his T4 and TSH levels were normal. On ultrasound thyroid scans, benign nodules were seen in 2 fuel handlers. TPO levels did not correlate with the TSH levels among the fuel handlers (r=-0.078, p=0.652). Inability to sustain an erection was reported by 35.5% fuel handlers which was significantly higher than controls who reported 5.6% (p=0.019). Premature ejaculation was reported by 27.9% of fuel handlers which was significantly higher than controls (p=0.023). The testosterone levels were significantly higher among fuel handlers compared to controls (p=0.048). The FSH and LH levels positively correlated with each other as expected in each subgroup and the total population (p<0.005). The TSH levels significantly negatively correlated with the testosterone levels among the fuel handlers. (r=- 0.338, p=.0.023). When the fuel handlers with premature ejaculation was considered the FSH, LH, Testosterone levels were not significantly different between the two groups, however the duration of employment was significantly longer among those reporting premature ejaculation. (p=0.024). **Conclusion:** There are thyroid and reproductive abnormalities among those exposed to fuel in an occupational setting. Disturbances to sexual functions may also be related to alteration of autonomic functions. Limiting exposure to fuel vapor will eliminate these detrimental effects and we propose self-service fuel pumps to be the best alternative to avoid occupational health hazards among fuel handlers.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Tildacerfont for the Treatment of Patients With Classic Congenital Adrenal Hyperplasia: Results From a 12-Week Phase 2 Clinical Trial in Adults With Classic CAH