1745 Mitochondrial Bioenergetics following Ozone Exposure in Rats Maintained on Coconut, Fish, and Olive Oil-Rich Diets

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Mitochondria are central regulators of energy homeostasis and may play a key role in mechanisms of neurodegenerative disorders and chemical-induced neurotoxicity. However, mitochondrial bioenergetic parameters have not been systematically evaluated under identical physiological conditions within multiple brain regions in rats maintained on different diets with subsequent exposure to environmental pollutants. In the present study, we measured complex I, complex II and complex IV enzymes using kits from Abcam® in the frontal cortex (FC), cerebellum (CER), hypothalamus (HYP), and hippocampus (HIP) of male Wistar Kyoto rats (N = 5 per group) that were fed either a normal diet (ND), or a diet enriched with fish oil (FO), olive oil (OO), or coconut oil (CO) starting at 4 weeks of age for 8 weeks followed by ozone (O_3) exposure (0 or 0.8 ppm O_3 for 4 h/day for 2 days). Immediately following O_3 exposure, rats were sacrificed, brain regions were dissected on ice, quick frozen on dry-ice, and stored at -80ºC until analysis. Complex I enzyme activity (NADH dehydrogenase, EC 1.6.5.3) was significantly lower in the CER, HIP, and HYP but not in FC of rats maintained on CO, FO and OO when compared to ND.O_3 exposure decreased complex I activity only in the CER of rats maintained on ND. Complex II enzyme activity (succinate dehydrogenase, EC 1.3.5.1) was significantly lower in FC and CER but not in HIP and HYP in rats maintained on all test diets when compared to ND. O_3 exposure had a significant effect in decreasing complex II enzyme activity in FC and CER of rats maintained on ND. Complex IV enzyme activity (cytochrome c oxidase, EC 1.9.3.1) was significantly lower in all brain regions of rats maintained on all test diets when compared to ND. The decrease by O_3 exposure was significant only in FC and HYP of rats maintained on ND. These results indicate that brain mitochondrial bioenergetics were significantly altered in rats maintained on different test diets and O_3 exposure had effects only on rats maintained on ND but not on rats maintained on FO, OO or CO. This suggests an interaction of diet and O_3 effects on brain mitochondrial bioenergetics parameters. *(This abstract does not necessarily reflect US EPA policy).*

1746 Corticosterone-Primed Neuroinflammatory Response to AChE Inhibitors Is Not Related to Brain Acetylcholine Concentration

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Roughly 30% of veterans from the 1991 Gulf War (GW) suffer from a persistent and heightened form of sickness behavior, which has been classified as Gulf War Illness (GWI). Previous GWI studies have suggested that exposure to acetylcholinesterase inhibitors (AChEIs) in-theater, such as sarin, a chemical warfare agent and irreversible AChEI, as well as other pesticides and insecticides, may have contributed to the symptomatology of GWI. In addition to exogenous chemical exposure, concomitant high physiological stress in-theater may have contributed to the initiation of the GWI phenotype. While inhibition of AChE leading to the accumulation of acetylcholine (ACh) will activate the cholinergic anti-inflammatory pathway, the exaggerated sickness behavior that is characteristic of GWI has been shown to be associated with neuroinflammation. To investigate the relationship between AChE inhibition and neuroinflammation, we used our previously established mouse model of GWI, which combines an exposure to a high physiological stress mimic, corticosterone (CORT), with GW-relevant AChEIs. Adult male C57BL/6J mice were exposed to CORT (400mg/L) in drinking water for 4 days. On the 5th day, mice were exposed to a single intraperitoneal (i.p.) dose of an AChEI: diisopropyl fluorophosphate (DFP; 4.0mg/kg), an irreversible AChEI and sarin surrogate, chlorpyrifos oxon (CPO; 8mg/kg), the active metabolite of chlorpyrifos, an irreversible AChEI insecticide used in-theater, and physostigmine (PHY; 0.5mg/kg), a reversible AChEI similar to pyridostigmine bromide, which was used as a prophylactic against nerve agents in-theater. After AChEI exposure, mice were sacrificed and ACh concentrations for cortex (CTX), hippocampus (HIP), and striatum (STR) were determined using hydrophilic interaction liquid chromatography (HILIC) with ultra-performance liquid chromatography (UPLC)-tandem-mass spectrometry (MS/MS). CORT pretreatment ameliorated the DFP-induced ACh increase in HIP and STR but not CTX. These effects were similar for CPO and PHY, but not as pronounced as DFP. qPCR of mRNA biomarkers for neuroinflammation revealed an exacerbated CORT+AChEI response, which does not correspond to ACh measured in the brain. This suggests that GWI may be due to off-target or secondary mechanisms of AChEI exposure via organophosphorylation of unknown biomolecular targets and not AChE inhibition.

1747 An 8-Week Educational Program Improves Mental Health of Individuals Exposed to Toxins

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Exposure to toxins has been associated with a depressed mood and major depression. We document the effect of common toxins on participants of an 8-week non-medical intervention in 5 continents. The educational program ran once a week for 8 weeks, the first 45 minutes participants viewed a DVD video followed by small group discussion together with weekly practical assignments. The program taught various healthy habits (diet, exercise, control of thoughts, etc). The Depression and Anxiety Assessment Test (registration TX 7-398-022) was applied at baseline and at the end. It assessed depression, demographics and asked about possible toxin exposure (intake of toxin-prone fish, lead exposure, etc). The program uses toxic exposure as one the many triggers of depression. The participants qualified as having the toxic exposure if they have: high lead levels, high mercury levels, high arsenic, bismuth, or other toxin levels or by having a high risk of exposure to these toxins. The depression was classified according to the DSM-5 [The Diagnostic and Statistical Manual of Mental Disorders Volume 5] into 4 categories as none (0-6), mild (7-10), moderate (11-19) or severe (20 or more). One session of the program educated participants about toxins and practical strategies to decrease exposure. Participants from 2008 to 2016 that finished the program were included, *n*=5757, in the retrospective study. Mean age 52.5 SD 15, 70% were females and 85.7% were Caucasians. At baseline those that had toxic exposure, *n*=1756, had a mean group depression of 13.2 SD 7.4 (SEM .17) mode 21, median 13 and those not exposed to toxins, *n*=4001, had a mean group depression of 11.9 SD 7.6 (SEM .12), mode 0, median 12. At baseline the toxin exposed group had 1.2 higher mean difference which was statistically significant ($p<.001$). By the end of the intervention, the group that had the toxin exposure at the beginning, had a mean group depression of 6.8 SD 6, mode 0, median 5 while the no toxin group had a mean group depression of 6.4 SD 5.9, mode 0, median 5. It seems toxin exposure increases depression levels in this population but the intervention is effective for both groups. The program is an effective tool to educate communities about toxins. A control group needs to be evaluated and a long-term follow-up is planned.

1748 Toxicity and Resilience Mechanisms in Luhmes 3D Model Exposed to Rotenone

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Introduction: Cellular "anastasis," Greek for resurrection, is a concept which is not implemented in current *in vitro* toxicological testing, however is relevant to mechanistic toxicology and adverse outcome pathways (AOPs). We hypothesize that not the primary hit, but the ability of the cells to cope with the stress (cellular recovery and resilience) determines organ selectivity. Alongside genetics, low dose exposures, can lead to disease in the long term. 3D cultures now allow for these studies *in vitro*. Objectives: Here, we expose an *in vitro* 3D dopaminergic model (3D LUHMES) to known mitochondrial toxicant rotenone, to compare molecular perturbations after primary hit (day 8 of differentiation), delayed effects 7 days after compound wash-out (day 15 of differentiation) and after secondary hit (day 16 of differentiation). We aim to study cellular recovery, delayed effects after low-dose exposures and molecular pathways involved in neurodegeneration such as Parkinson's disease. Methods: We treated organoids with 50 and 100 nM rotenone and analyzed perturbations in transcriptome on day 8 and day 15. We further studied functional endpoints such as DNA damage and repair, neurite outgrowth, ATP level, mitophagy, alpha-synuclein accumulation and dopamine production at both time points. Results: We analyzed acute and delayed response of 3D LUHMES to rotenone to identify early pathways of toxicity vs. defense; recovery vs. adverse outcome. This encompasses, for example, the early activation of the Notch signaling pathway vs. later perturbations in energy metabolism, oxidative stress and delayed induction of α-synuclein (PD hallmark) expression. Increased DNA damage and repair were observed after compound withdrawal in 100 nM treated samples. In the same experiments, pro-apoptotic mir-16 was induced only on day 15 (delayed); while miR-7, known to play a neuroprotective role in Parkinson's disease models, was significantly downregulated after acute exposure and upregulated on day 15 (reversible). Perturbations in ATP production and neurite outgrowth were concentration-dependent and partial reversible after rotenone withdrawal. Conclusion: This study illustrates on a molecular level, how chemical exposure alters cellular responsiveness, including the induction of protective means, but also molecular scars possibly associated with late hazard manifestations and disease manifestation.


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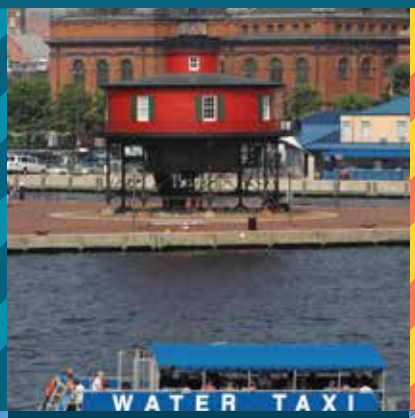

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