Evaluation of Mitochondrial Nitric Oxide Synthase (iNOS) upon Exposure to PAHS

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Abstract: Nitric oxide (NO) plays an important role in the mitochondrial function and regulation including energy metabolism, signaling and apoptosis. It has been recently found that mitochondrial biogenesis and function are enhanced by nitric oxide synthase. This experiment was planned to check the status of NO synthase in mitochondrial dysfunction resulting from the exposure to polycyclic aromatic hydrocarbons (PAHs). Human breast cancer MCF 7 cells were cultured for 3 days, then exposed to 6 different treatments: Dimethyl sulfoxide, Acetonitrile, Benzo(a)pyrene high and low doses, and polycyclic aromatic hydrocarbons high and low doses. After 24hrs of exposure, MTT assay was performed in a 96 well plate. The exposure medium obtained from the cells after 24hrs were used for lactate dehydrogenises (LDH) assay. Proteins were extracted by M-per protein extractions and protein assay was performed to examine the amount of protein to be loaded in the gel electrophoreses for immunolocalization of Nitric Oxide Synthase-Inducible (iNOS). In both assays it was observed that the high dose (7µM) of PAHs damaged mitochondrial functions in MCF 7 cells leaving less viable cells in the sample. However, the immunolocalization of iNOS by western blot was performed, followed by the adding of a household protein: ßeta-actin to the blot, which also shows that PAH high dose has higher reaction on inducible nitric oxide in mitochondrial. This shows that the proposed mechanism for this experiment was met.