The Organophosphate Pesticide Methyl-Parathion Modulates the Expression of DNA Methylation-Demethylation Genes Through Oxidative Stress in Mice Testicular Cells

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Introduction

DNA methylation-demethylation **(DNA-M/D)** is an epigenetic mechanism, which is associated with gene expression. DNA methylation involves the covalent transfer of methyl groups to cytosine from cytosine-guanine **(CpG)** sites, forming 5-methylcytosine **(5-mC);** this process is catalyzed by DNA-methyltransferases **(DNMTs)[1].** Active DNA demethylation, catalyzed by ten-eleven translocation enzymes **(TETs),** consists in the sequential oxidation of 5-mC to finally be repaired to cytosine [(2)] The regulation of this process is not fully known; however, it has been proposed that reactive oxygen species **(ROS; endogenous or exogenous)** can regulate DNA-M/D [(3)]. The organophosphate pesticide methyl-parathion **(Me-Pa),** despite is highly toxicity, is employed in developing countries and it produces oxidative damage in macromolecules of sperm cells [(4,5),] as well as DNA alkylation. Recently, we reported that Me-Pa exposure generates promoter-specific hypermethylation in antioxidant response and DNA repair genes in sperm cells [(6)] , but the mode of action is unknown.

Objective

To evaluate the *DNMTs* and *TETs* expression and methyl-purine DNA glycosylate **(*MPG;* alkylation repair gene)** expression in testicular cells of mice exposed to Me-Pa (6 mg/kg/day/5 days) and co-exposed with Me-Pa (same dose)-Vitamin E (50 mg/kg/day/5 days) to evaluate ROS participation.

Procedure

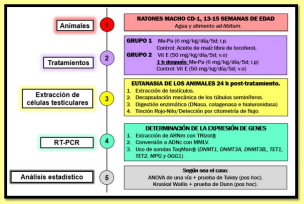

Figure 1. Schematic ilustration of the procedure for this work.

Results

ROS generated by Me-Pa exposure increased the DNA methylation genes expression of sperm cells

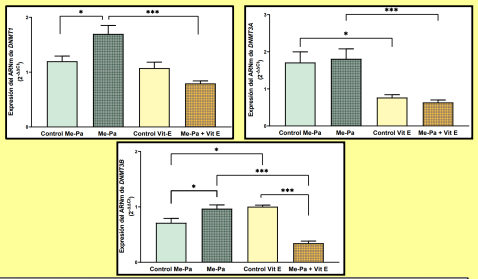

Figure 2. mRNA expression levels of A) *DNMT1,* B) *DNMT3A* and C) *DNMT3B* in testicular cells after Me-Pa exposure and Me-Pa + Vit-E co-exposition. Bars represent the mean ± SEM. Samples were analyzed by triplicated. Significant difference (* $p < 0.05$; *** $p < 0.001$) between groups according to the ANOVA test and Tukey *pos-hoc* test. n=3-5 controls, n= 4-5 Me-Pa exposed and n=5-6 for co-exposed group.

Results (cont)

ROS generated by Me-Pa exposure increased the DNA methylation genes expression of sperm cells

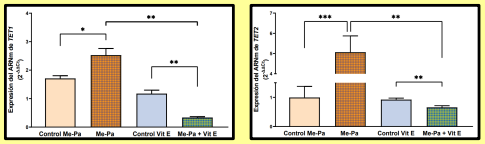

Figure 3. mRNA expression levels of A) *TET1* and B) *TET2* in testicular cells after Me-Pa exposure and Me-Pa + Vit-E co-exposition. Bars represent the mean ± SEM. Samples were analyzed by triplicated. Significant difference (* $p < 0.05$; *** $p < 0.001$) between groups according to the ANOVA test and Tukey *pos-hoc* test. n=3-5 controls, n= 4-5 Me-Pa exposed and n=5-6 for co-exposed group.

Me-Pa exposure modulated the alkylated bases repair gene expression *MPG*

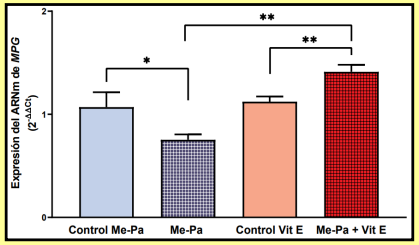

Figure mRNA expression level *MPG* in testicular cells after Me-Pa exposure and Me-Pa + Vit-E co-exposition. Bars represent the mean ± SEM. Samples were analyzed by triplicated. Significant difference (* $p < 0.05$; ** $p < 0.001$) between groups according to the ANOVA test and Tukey *pos-hoc* test. n=3-4 controls, n= 5 Me-Pa exposed and n=6 for co-exposed group.

Conclusions

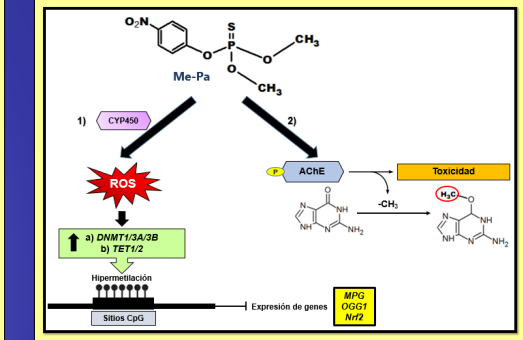

Figure 5. Toxicity nechanism of Me-Pa in testicular cells from mice exposed to Me-Pa. 1) Repeated exposure (6 mg/kg/day/5 days) to Me-Pa generates ROS in its metabolism (mediated by CYP450), which regulates a) *De novo* (*DNMT3A* y *DNMT3B)* and maintenance (*DNMT1*) *DNMTs (*DNA methylation*)*, b) *TET1* and *TET2 (*DNA demethylation*)*. Both alterations could to explain the hypermethylation phenomenon suggested, in this work, in *MPG* promoter (alkylated-bases gene repair) and as we previously reported in *OGG1* (oxidized-bases gene repair) and Nrf2 (antioxidant-response gene) [(6)] , which could mean their silencing. **2)** Me-Pa exposure could generates direct DNA alkylations, probably due the "release" of methyl groups, possibly for the aging of acethylcholinesterase (AChE)

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