Oxidants but Not Alkylating Agents Induce Rapid mtDNA Loss and Mitochondrial Dysfunction

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Mitochondrial DNA (mtDNA) is essential for proper mitochondrial function and encodes 22 tRNAs, 2 rRNAs and 13 polypeptides that make up subunits of complex I, III, IV, in the electron transport chain and complex V, the ATP synthase. Although mitochondrial dysfunction has been implicated in processes such as premature aging, neurodegeneration, and cancer, it has not been shown whether persistent mtDNA damage causes a loss of oxidative phosphorylation. We addressed this question by treating mouse embryonic fibroblasts with either H2O2 or the alkylating agent methyl methanesulfonate (MMS) and measuring several endpoints; these include mtDNA damage and repair rates using QPCR, levels of mitochondrial- and nuclear-encoded proteins using western analysis, and a pharmacologic profile of mitochondria using the Seahorse Extracellular Flux Analyzer. We show that a 60 minute treatment with H2O2 causes persistent mtDNA lesions, mtDNA loss, decreased levels of a mitochondrially-encoded subunit of complex I, a loss of ATP-linked oxidative phosphorylation and a loss of maximal respiration capacity. In contrast, a 60 minute treatment with 2 mM MMS causes persistent mtDNA lesions but no mtDNA loss, no decrease in levels of a mitochondrially-encoded protein, and no mitochondrial dysfunction. This study is the first to measure MMS-induced lesions and the effects of MMS on mitochondrial function, and our results suggest that persistent mtDNA damage is not sufficient to cause rapid mitochondrial dysfunction and loss of mtDNA.