MOLECULAR AND CELLULAR EFFECTS OF LOW-DOSE CHRYSOTILE ASBESTOS EXPOSURE IN HUMAN LUNG FIBROBLASTS

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Background: Chrysotile asbestos, the predominant serpentine mineral fiber still in industrial use, remains classified as a Group 1 human carcinogen by the International Agency for Research on Cancer. Although historically regarded as less hazardous than amphibole fibers, chrysotile exposure continues to pose substantial occupational and environmental risks, particularly in Kazakhstan, one of the world's largest producers. Mounting evidence indicates that chrysotile induces oxidative stress, genomic instability, and cell death—critical determinants in the pathogenesis of pulmonary fibrosis and lung cancer. Notably, the release of circulating cell-free mitochondrial DNA (cf mtDNA) has emerged as a robust biomarker of mitochondrial dysfunction and apoptotic signaling, yet its role in asbestos-related cytotoxicity remains insufficiently characterized.

Materials and Methods: Human lung fibroblasts (MRC5) were exposed to chrysotile asbestos at 2.5, 5, and 10 μg/cm². Intracellular reactive oxygen species (ROS) were quantified using CM-H2DCFDA fluorescence. Genotoxicity was assessed by alkaline comet assay, cytotoxicity by MTT assay, and cf mtDNA levels in culture supernatants by quantitative PCR. Statistical significance was determined by one-way ANOVA or Kruskal–Wallis test, with $p \leq 0.05$ considered significant.

Results: Chrysotile induced a pronounced, dose- and time-dependent increase in ROS, peaking at 24 h. DNA integrity was severely compromised, with comet assay revealing tail DNA% up to 38% at 10 μg/cm² versus <1% in controls. Cell viability declined progressively, reaching 65.9% after 48 h at the highest concentration. Strikingly, cf mtDNA levels exhibited exponential accumulation, peaking at 1.37×10^{16} copies/mL after 48 h, consistent with extensive mitochondrial damage and apoptotic/necrotic cell death.

Conclusion: These findings provide compelling mechanistic evidence that chrysotile asbestos provokes oxidative stress, DNA fragmentation, and loss of cell viability, accompanied by a marked release of cf mtDNA. The latter constitutes a promising biomarker of asbestos-induced mitochondrial dysfunction and programmed cell death. Collectively, the data refute the long- standing assumption of chrysotile safety and underscore its pathophysiological significance in the initiation of asbestos-related lung disease and carcinogenesis.

Key words: chrysotile asbestos, reactive oxygen species; oxidative stress; DNA damage; cell viability; cell-free mitochondrial DNA (cf mtDNA).

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