

论文

多壁碳纳米管诱导A549细胞氧化应激与去极化线粒体膜电位

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摘要:

以人肺上皮细胞系A549为模型细胞, 探讨多壁碳纳米管的细胞毒性效应及其机制. A549细胞暴露于不同浓度(0~300 $\mu\text{g/mL}$)的多壁碳纳米管后, 用MTT比色法检测细胞活力和Hoechst 33342染色法观察细胞形态; 用活性氧(ROS)敏感探针2',7'-二氯荧光素二乙酸酯(DCFH-DA)结合流式细胞仪检测细胞内ROS水平; 用荧光探针JC-1结合激光共聚焦显微镜检测细胞线粒体膜电位 $\Delta\psi_m$ 的变化; 用免疫荧光和蛋白印迹法检测细胞氧化应激敏感蛋白血红素氧合酶-1(HO-1)的表达水平. 结果表明, 多壁碳纳米管可引起A549细胞活性降低、细胞内活性氧ROS过量产生以及谷胱甘肽GSH含量下降, 诱导细胞氧化应激效应; 抗氧化剂N-乙酰半胱氨酸(NAC)抑制多壁碳纳米管诱导的A549细胞内ROS的产生. 多壁碳纳米管处理A549细胞2 h后, 诱发细胞线粒体膜电位下降; 多壁碳纳米管诱导细胞氧化应激的同时伴有适应性应激蛋白HO-1的上调表达. 结果表明, 细胞氧化应激和线粒体膜电位去极化可能是多壁碳纳米管诱导A549细胞毒性效应的重要机制.

关键词: 多壁碳纳米管 线粒体膜电位 氧化应激 血红素氧合酶-1

Multi-walled Carbon Nanotubes Exposure Induces Oxidative Stress and Depolarizes Mitochondrial Membrane Potential in Cultured A549 Cells

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Abstract:

The present study was undertaken to determinate the cytotoxicity exhibited via multi-walled carbon nanotubes(MWCNTs) in human A549 lung epithelial cells as a model system. Cell mortality was measured by methyl thiazolyl tetrazolium(MTT) assays. The morphological changes of A549 cells were examined by Hoechst 33342 staining. The level of reactive oxygen species(ROS) was detected by means of flow cytometry analysis with a redox-sensitive fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA).The mitochondria membrane potential was observed by a confocal laser-scanning microscope with JC-1 fluorescence. The heme oxygenase-1(HO-1) protein expression was analyzed by immunofluorescence and Western blotting. The result reveal that treatment of A549 cells with MWCNTs lead to loss of cell viability, ROS production, glutathione depletion and decline in mitochondrial membrane potential. MWCNTs were potent toward inducing cellular heme oxygenase-1(HO-1) expression, a sensitive biomarker for oxidative stress. Our observations suggest that MWCNTs exert toxicity by increasing oxidative stress, decreasing the mitochondrial membrane potential in cultured A549 cells.

Keywords: Multi-walled carbon nanotubes Mitochondrial membrane potential Oxidative stress Heme oxygenase-1

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