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论著

曲霉菌素诱导人胚肾细胞毒性机制

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摘要 目的 研究曲霉菌素诱导人胚肾细胞毒性作用机制。方法 结晶甲紫法用于细胞存活率研究。琼脂糖凝胶及Burton法研究细胞核DNA断裂片段。以水解特异性底物Ac-DEVD-AMC活性为研究指标，测定胞浆半胱天冬酶(caspase)-3类蛋白酶活性。采用蛋白质印迹法检测细胞半胱天冬酶-3蛋白表达。采用荧光标记探针、流式细胞仪技术研究细胞核DNA核型及细胞活性氧的产生。结果 曲霉菌素浓度依赖性地诱导人胚肾细胞凋亡，最大效应浓度为1.0 mg·L^{-1}。BAF, 半胱天冬酶-3蛋白抑制剂和*N*-乙酰半胱氨酸(活性氧抑制剂)能显著性抑制曲霉菌素诱导人胚肾细胞凋亡作用。结论 半胱天冬酶类及活性氧调节曲霉菌素诱导的人胚肾细胞凋亡。

关键词 曲霉菌素 凋亡 细胞, 人胚肾 半胱天冬酶类 活性氧

分类号 R99

Mechanism of cytotoxicity of human embryonic kidney cells induced by gliotoxin

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Abstract

AIM To study the mechanism underlying gliotoxin-induced cytotoxicity of human embryonic kidney (HEK) cells. METHODS Crystal violet assay was used to determine cell viability. DNA fragmentation of HEK cells was measured based on Burton's method. The activity of caspase-3-like proteases was measured as increases in hydrolysis of fluorogenic tetrapeptide substrate, Ac-DEVD-7- amino-4-methylcoumarin and caspase-3 protein abundance was observed by Western blot. Based on fluorescence probe label method, DNA content and reactive oxygen species (ROS) of HEK cells were detected by flow cytometry. RESULTS Gliotoxin induced HEK cell death in a concentration-dependent manner within 0.4—1.0 mg·L^{-1}. Under gliotoxin treatment at 1.0 mg·L^{-1}, cell membrane of HEK cells kept intact associated with hypodiploid nuclei and DNA fragmentation which suggested gliotoxin killed HEK cells *via* apoptosis. Boc aspartyl (OMe)-fluoromethylketone (BAF) and z-DEVD.fmk, commonly used as caspase-3-like proteases inhibitor, significantly abolished gliotoxin-induced cell death at 100 and 200 μmol·L^{-1}, respectively, suggesting the cytotoxicity induced by gliotoxin was mediated by caspases. *N*-acetylcysteine concentration-dependently attenuated the HEK cells death induced by gliotoxin, significantly inhibited the generation of ROS of HEK cells upon exposure with gliotoxin, which indicated that ROS was involved in the cytotoxicity of HEK cells induced by gliotoxin. CONCLUSION Gliotoxin-induced cytotoxicity of HEK cells proceeded *via* apoptosis, which was mediated by caspases and ROS.

Key words gliotoxin apoptosis cells human embryonic kidney caspases reactive oxygen species

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