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论著

依非韦伦的肝细胞毒性作用及对蛋白质表达谱的影响

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摘要 **目的** 探讨依非韦伦对肝细胞的毒性作用及对蛋白质表达谱的影响。**方法** 人肝癌细胞系Huh7中分别加入依非韦伦1.25，2.5，5和10 mg·L^{-1}，培养5 h后采用原位比色法测定细胞内活性氧类（ROS）的含量；依非韦伦2.5 mg·L^{-1}与细胞作用5 h后，用膜联蛋白-Ⅴ/碘化丙啶细胞凋亡检测试剂盒染色。用流式细胞仪测定细胞凋亡；依非韦伦2.5 mg·L^{-1}处理细胞5 h，获得全细胞蛋白质进行二维凝胶电泳，采用ImageMaster软件分析差异蛋白质点，应用纳升级液相色谱串联电喷雾离子阱质谱进行差异蛋白质鉴定。**结果** 随着依非韦伦浓度的增加，细胞内ROS的含量逐步升高（r=0.9740，P<0.05）。依非韦伦2.5 mg·L^{-1}与细胞作用5 h后，与正常对照组比较，细胞凋亡百分率无显著变化，但有7种蛋白质表达量显著降低，其中线粒体热激蛋白75和抗氧化蛋白1的表达量分别降低了76.7%和85.5%；抗胰蛋白酶及其S突变体、细胞角蛋白9、剪接体相关蛋白和磷酸丙糖异构酶的表达被完全抑制。**结论** 依非韦伦对Huh7细胞具有明显的细胞毒性，可能通过调节热激蛋白75和抗氧化蛋白1等的表达影响线粒体的功能，最终导致肝毒性的发生。

关键词 依非韦伦 肝细胞 毒性 活性氧类 蛋白质组学

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Proteomic analysis and hepatotoxicity of efavirenz in hepatocytes

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Abstract

OBJECTIVE To investigate the hepatotoxicity of efavirenz (EFV) in hepatocytes and its impact on proteome profile. METHODS The reactive oxygen species (ROS) in cells were detected by colorimetry analysis after human liver cancer Huh7 cells were cultured with EFV 1.25，2.5，5 and 10 mg·L^{-1} for 5 h, respectively. Cell apoptosis was analyzed by flow cytometry following Annexin-Ⅴ/propidium iodide dying after Huh7 cells were incubated with EFV 2.5 mg·L^{-1} for 5 h. The proteins extracted from cells treated with EFV 2.5 mg·L^{-1} or untreated control cells were separated by two-dimensional gel electrophoresis, and the differently expressed proteins in normal control and EFV 2.5 mg·L^{-1} groups were analyzed using ImageMaster software and identified by online reversed-phase nano-flow liquid chromatography coupled with ion trap mass spectrometry. RESULTS Compared with normal control cells, the ROS content in EFV groups significantly increased (P<0.05), and there was a good concentration-effect relationship（r=0.9740，P<0.05）. After EFV 2.5 mg·L^{-1} treatment for 5 h, the apoptosis percentage had no significant change, but expression level of seven proteins decreased significantly, among which mitochondrial heat shock protein 75 and antioxidant protein 1 were down-regulated 4.3 and 7.7 times by EFV, respectively. Alpha-1-antitrypsin, the S variant of alpha-1-antitrypsin, cytokeratin 9, pre-mRNA-splicing factor and triosephosphate isomerase were completely inhibited by EFV. CONCLUSION EFV has hepatotoxicity, and EFV might regulate mitochondrial function through decreasing the expression of MTHSP75 and antioxidant protein 1 in mitochondria, which may contribute to its hepatotoxicity.

Key words efavirenz hepatocytes toxicity reactive oxygen species proteomics

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