鎘及汞造成人類正常肺細胞 MRC-5 死亡途徑的探討

Studies on the Pathway of Cell Death Triggered by Cadmium and Mercury in Human Normal Lung Cell MRC-5

中文摘要

流行病學證據顯示: 鎘及汞污染，可能導致肺部傷害及病變，但其機轉目前尚不清楚。本論文以人類正常肺胚胎細胞(MRC-5)爲細胞模式，探討鎘和汞傷害細胞的機制。以下列方法偵測細胞凋亡,首先利用 Hoechst staining 及核磁共振分析 1H NMR 在 1.3 ppm (methylene) 及 0.9 ppm (methyl) 的變化，探討鎘及汞處理下，MRC-5 細胞死亡型態，續以流式細胞儀 PI 染色及 Annexin V/PI 雙染色，定量分析細胞凋亡及壞死，以 100 mM Cd 處理細胞，大約 40 % 細胞凋亡(apoptosis)，另有 20 % 細胞壞死(necrosis)，以 100 mM Hg 處理細胞，有 70 % 細胞壞死， 6 % 細胞凋亡。本實驗室之前的研究成果指出，利用 dichlorodihydrofluorescein diacetate (DCFH-DA) 發現加入鎘後 H2O2 增加三倍，故推論鎘造成 MRC-5 細胞凋亡可能與活性氧分子 reactive oxygen species (ROS) 有密切關係。據此，本論文續以抗氧化劑，包括 N-acetylcysteine (NAC)、4,5-dihydroxy-1,3 benzene-disulfonic acid (tiron) 以及 mannitol 處理細胞，發現可抑制鎘之毒性，證明 ROS 參與鎘之細胞毒性。細胞內最主要生成 ROS 的胞器是粒線體，利用流式細胞儀以 5,5’,6,6’-tetrachloro-1,1’3,3’-tetra-ethylbenzimidazolylcarbocyanime iodide (JC-1)染劑觀察粒線體膜電位變化，發現加入鎘之後，隨時間變化，粒線體膜電位有去極化的趨勢，利用粒線體電子傳遞鏈抑制劑，如: rotenone、 oligomycin A 以及粒線體 permeability transition pore 抑制劑，包括 cyclosporin A 及 aristolochic acid 均可抑制鎘所造成粒線體膜電位去極化，進而抑制其細胞毒性。我們推論鎘可直接或間接作用於粒線體，導致胞內氧化壓力增加，進而造成細胞毒性。爲了瞭解鎘造成細胞凋亡的機制，我們利用西方墨點法發現 pro-caspase 3 未被活化，且加入 caspase 抑制劑 z-VAD-fmk 並無法抑制鎘所造成細胞毒性，推測鎘造成 MRC-5 細胞凋亡是屬於 caspase-independent pathway。此外，Apoptosis-inducing factor (AIF) 位於粒線體內，當接受到死亡訊息，則由粒線體轉位 (translocate) 至細胞核，造成染色體濃縮，且使 DNA 形成大的片段化(~50 kb) ，導致細胞進行 caspase-independent 凋亡，利用免疫螢光法，我們發現鎘處理後，胞內 AIF 有往核集中的趨勢。綜合以上結果，我們推論鎘可能直接或間接作用於正常人類肺胚胎細胞 (MRC-5)的粒線體產生 ROS，並釋放 AIF，導致細胞進行 caspase-independent 凋亡，而汞處理細胞則導致細胞走向壞死。

英文摘要
Epidemiological evidence has suggested that exposure of the human to cadmium (Cd) and mercury (Hg) might cause pulmonary damages. However, the mechanism has remained unclear. A normal human fetal lung fibroblast cell line (MRC-5) was used as a cell model to investigate the mechanism of cell death triggered by Cd or Hg. Several methods were employed to elucidate apoptosis and necrosis of MRC-5 cells after treatment of 100μM Cd or Hg, respectively; including cytohistochemistry analysis with Hoechst staining, flow cytometric assay with propidium iodide (PI) single staining as well as annexin V plus PI double staining and 1H NMR to analyze the spectral intensity ratio of methylene (1.3 ppm) to methyl (0.9 ppm) resonances. We have previously demonstrated that H2O2 was elevated three folds after treatment of Cd by using dichlorodihydrofluorescein diacetate (DCFH-DA) as a detection agent. These results suggested that reactive oxygen species (ROS) might play pivotal roles during apoptosis and cytotoxicity induced by Cd. Following this line, the antioxidant compounds, such as N-acetylcysteine (NAC), 4, 5-dihydroxy-1, 3 benzene-disulfonic acid (tiron) and mannitol were used to rescue MRC-5 cells from the deleterious effect of ROS. The results showed that the ROS-generating organelle, mitochondrion, was depolarized and participated in Cd-induced apoptosis as revealed by 5,5′,6,6′-tetrachloro-1,1′3,3′-tetra-ethyl benzimidazolyl carbo -cyanime iodide (JC-1)/flow cytometric analysis. In addition, rotenone and oligomycin A are inhibitors of electron transport chain (ETC), which could decrease the cytotoxicity of Cd and reduce the percentage of apoptotic cells. Pre-treatment with cyclosporin A and aristolochic acid (inhibitor of mitochondrial permeability pore formation) significantly reduced the mitochondrial membrane potential, and attenuated the signs of apoptosis such as DNA fragmentation by detection with PI staining. This result indicated that mitochondria might play an arbitrator for Cd-triggered apoptosis of MRC-5 cells. On the other hand, we used the immunoblot to demonstrate that caspases was not activated and its substrate poly (ADP-ribose) polymerase (PARP) was not cleaved in Cd-indicated apoptotic cells. Moreover, the pan-caspase inhibitor, z-Val-Ala-Asp- (OME)- fluoromethylketone (z-VAD- fmk), could not prevent Cd-induced cell death, which implied a caspase-independent apoptotic pathway. Recent reports prove that apoptosis-inducing factor (AIF) is normally confined to the mitochondrial membrane space and translocates through the outer mitochondrial membrane to the nucleus in a caspase-independent apoptotic pathway. By indirect-immunofluorescence, AIF was observed to translocate from mitochondria to the nucleus after Cd treatment. In conclusion, our results suggest that Cd triggered a caspase-independent apoptotic pathway through mitochondria and ROS mechanism in human normal lung cell. In contrast,

Hg induced cell death by necrosis.