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• 中文摘要

鎘爲環境汙染物，且已爲 International agency for research on cancer (IARC)認証爲一級致癌物。經由不同投與路徑所吸收的鎘會囤積於腎臟中，導致病人腎絲球過濾失常，腎功能缺損進而死亡，但是其分子機制則尚未清楚，而本計劃則利用腎間質細胞(mesangial cell)以偵測鎘腎毒性的分子機制。結果顯示，經由鎘處理的腎間質細胞會產生劑量(IC50=0.72 μM)及時間依存的細胞凋亡，且其過程中伴隨著粒線体膜電位的下降。而利用 Fluo-3 AM 染劑偵測鈣離子濃度變化，結果証實鎘會誘使細胞質中的鈣離子的濃度增加，且 BAPTA-AM 亦有效的抑制鎘所導致的細胞凋亡。內質網是細胞中最主要的鈣離子調節胞器，且 IP3R 的抑制劑可以有效的抑制鎘的腎毒性，而相對的，calcineurin 的抑制劑則會促使細胞內鈣離子上昇，進而增加鎘的細胞毒性，因此推測經由內質網釋放所導致的胞內鈣離子增加是鎘毒性中的主要致死因子。且經由 western blot 實驗，我們亦發現內質網壓力的標誌蛋白質(GADD153)有大量增加及 caspase 12 的活化，而 BAPTA-AM 則可以抑制此現象，並且 pan-caspase 抑制劑亦足以抑制其細胞毒性。綜合以上所見，我們推論鎘可經由促使內質網的鈣離子釋放，進而產生內質網壓力及 caspase 的活化，最終導致腎間質細胞凋亡。

• 英文摘要

Cadmium is an industrial and environmental pollutant that affects many organs in humans and other mammals. The kidney, a major organ involved in the elimination of systemic Cd, is especially sensitive to the cytotoxicity of cadmium, resulting in Fanconi's syndrome, proteinuria, calciuria and phosphaturia. However, the molecular mechanisms of Cd-induced nephrotoxicity are unclear. In this study, we showed that Cd-induced mesangial cells (MES-13) underwent apoptosis as dose (IC50= 0.72 .mu.M) and time course dependent manners, which accompanied with the collapse of mitochondria. Using Fluo-3 AM, the intracellular concentration of calcium ([Ca2+]) was detected to elevate as time elapsed after Cd treatment. Co-treatment with BAPTA-AM, a Ca2+ chelater, is able to suppress Cd-induced apoptosis significantly. Calcineurin is a cytosolic phosphatase, which could dephosphorlyate the IP3R to prevent

releasing of calcium from endoplasmic reticulum (ER). Cyclosporine A, a calcineurin inhibitor, increased the [Ca2+]i and the percentage of Cd-induced apoptosis. However, the IP3R inhibitor, 2-APB, is able to partially modulate the Cd cytotoxicity. These results lead us to suggest that the increase of [Ca2+]i is one of the crucial roles in Cd-induced apoptosis in mesangial cells. Following this line, we further detected the ER stress after Cd treatment since ER is one of the major organelles for calcium storage. After exposure of Cd, GADD153, a hallmark of ER stress, was up-regulated (4 h exposure), followed by activation of ER-specific caspase-12 and its downstream molecule caspase-3 (16 h exposure). The pan caspase inhibitor, Z-VAD, and BAPTA-AM could reverse the Cd-induced cell death and ER stress, respectively. Therefore, we concluded that nephrotoxity of cadmium on the mesangial cells is through ER-mitochondria pathway and calcium oscillation plays a pivotal role.