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摘要(中)	輔仁大學八十八學年度第二學期碩士論文摘要 系(所)別:食品營養學系 研究生:黃淑美 論文名稱:同半胱胺酸硫化內酯誘發哺乳動物細胞傷害及相關營養素對細胞傷害調節之探討 指導教授:許瑞芬 博士 摘 要 同半胱胺酸對血液循環系統之細胞傷害機制已有詳細的研究，但對於其它局部組織細胞之影響則未知。本研究發現病理濃度的同半胱胺酸衍生物(同半胱胺酸硫化內酯) 會誘發人類前骨髓血癌 HL-60 細胞程式凋亡。同半胱胺酸硫化內酯誘發 HL-60 之程式凋亡，造成磷脂絲胺酸露出細胞表面的比例增加、具有低染色體套數 DNA 的細胞增加，及細胞核內 DNA 呈現 180~200 鹼基對大小的斷裂形式。同半胱胺酸硫化內酯對細胞膜及核 DNA 造成的程式凋亡，隨著劑量及處理時間的增加而增加。同半胱胺酸硫化內酯促使細胞內活性氧化物種 H2O2 於凋亡特徵出現前增加了 2~6 倍，隨即活化程式凋亡執行蛋白 caspase-3 的活性。Caspase-9 抑制劑延遲同半胱胺酸硫化內酯引發的 caspase-3 活化，但無法完全抑制凋亡的發生。過氧化氫?可完全清除 HL-60 細胞內的 H2O2，抑制 caspase-3 的活化，防止同半胱胺酸硫化內酯處理導致 DNA 受損及細胞膜破壞的發生，但超氧化物歧化?則無明顯效果。抗氧化營養素 NAC、Vit. C 可部分抑制同半胱胺酸硫化內酯誘發的 H2O2 生成、caspase-3 活化和凋亡，Vit. E 可減少低染色體套數 DNA 的比例，但外加同半胱胺酸相關營養素(葉酸、維生素 B6 和 B12)則沒有明顯抑制效果。同半胱胺酸硫化內酯對中國大頰鼠卵巢細胞(CHO)只有產生遲滯生長的現象，而且對葉酸代謝不同的 CHO 之毒性程度不同，以粒線體葉酸代謝異常的 GlyA 反應最明顯。由這些結果可知，不同類型細胞對同半胱胺酸硫化內酯的細胞染色體傷害毒性之敏感度不同。同半胱胺酸硫化內酯可能是藉由產生過氧化氫，經 caspase-9 途徑而引起 caspase-3 活化，進而誘發 HL-60 細胞凋亡。營養素中以 NAC、Vit. C 和 Vit. E 對同半胱胺酸硫化內酯誘發的凋亡有較佳的抑制作用。外加葉酸、維生素 B6 和 B12 沒有作用，但細胞內葉酸的代謝可調節同半胱胺酸硫化內酯的毒性。 關鍵字：同半胱胺酸硫化內酯、細胞凋亡、過氧化氫、caspase-3、CHO、HL-60
摘要(英)	Modulation of Antioxidants and Folate Related Nutrients on Homocysteine Thiolactone Induced Apoptosis in Mammalian Cell Lines Shu-Mei Huang Abstract The cytotoxicity of homocysteine on localized tissues other than from circulatory system is not well established. The present study revealed that human promyeloid leukemia HL-60 cells were induced apoptosis by homocysteine derivatives (HcyT) treatment at pathological concentrations. Apoptosis induced by HcyT was characterized by increased phosphatidylserine (PS) exposure on the cell membrane surface, increased apoptotic cells with hypoploid DNA contents, and internucleosomal DNA fragmentation, all of which occurred in a time- and dose-dependent manner. HcyT treatment also significantly increased intracellular reactive oxygen species H2O2 and caspase-3 activity, which was accompanied by the

	appearance of apoptotic features. Addition of caspase-9 inhibitor to HcyT-treated HL-60 cells delayed caspase-3 activation, but could not completely prevent cells from apoptotic damage. Preincubation of HcyT-treated HL-60 cells with catalase completely scavenged intracellular H2O2, thus inhibiting caspase-3 activity and protecting cells from apoptotic DNA damage and membrane injury. Conversely, antioxidants of NAC, Vit C and Vit E partially inhibited HcyT-induced H2O2 production、caspase-3 activation and apoptosis. Neither SOD nor Hcy-metabolizing nutrients (folate, vitamin B6 and B12) had the significant effects on the prevention of HcyT-treated HL-60 cells from apoptotic damage. Chinese hamster ovary cells (CHO) were less sensitive to HcyT-induced cytotoxicity than HL-60 cells. Cells with mitochondrial defects were more sensitive to HcyT-induced cell growth arrest than cells with cytosol defects. Taken together, these results suggest that different cell types have different susceptibility to HcyT toxicity. HcyT treatment induced apoptosis in HL-60 cells through H2O2 generation, and caspase-3 activation. Antioxidants of NAC、Vit C and Vit E could partially reduce HcyT-exerted apoptotic damage. Addition of folate、Vit B6 and B12 had no significant effects on HcyT-induced apoptotic damage, yet intercompartment folate metabolism could modulate HcyT-induced cytotoxicity. Key words: homocysteine thiolactone、apoptosis、H2O2、caspase-3、CHO、HL-60
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