吳茱萸鹼誘導惡性神經膠質細胞瘤 U87-MG 細胞死亡機制之研究

Effects of Evodiamine on the Cell Death in Human U87MG Glioma Cells

中文摘要

惡性膠質細胞瘤爲原發性腦瘤，以手術完全切除事實上相當困難，因此，常另外以化學治療爲輔助療法。細胞自噬(autophagy)爲細胞內蛋白質或胞器分解再利用的過程，被歸類爲 type II 細胞死亡型式。許多證據顯示，抗癌藥之細胞毒殺作用與引發細胞自噬有關。吳茱萸鹼(evodiamine)是由中草藥吳茱萸萃取出來具有生物活性之生物鹼。已有文獻証實，evodiamine 可誘導癌細胞經由細胞凋亡(apoptosis)型式死亡，對於 autophagy 之角色，至今尚未完全了解。爲提供腦瘤之新藥開發一條新的方向，因此本論文研究 evodiamine 誘導人類惡性神經膠質細胞瘤 U87-MG 細胞死亡的型式，並探討其中可能參與之訊息傳遞因子。

本研究利用 MTT assay 分析 evodiamine 對 U87-MG 細胞生長之影響。實驗結果顯示，evodiamine 對 U87MG 細胞存活率有明顯的抑制效果($p<0.001$)。利用 acridine orange 染劑搭配 flow cytometry 偵測 acidic vesicular organelle (AVO)含量，代表 autophagy 之百分比，結果發現 evodiamine 處理細胞 24 小時可達最高 autophagy 百分比(42.3 ± 7.5 %, $p<0.001$)，48 小時下降至 20.4 ± 5.5 % ($p<0.01$)，利用 Western blot 亦可觀察到 autophagy 過程中 LC3 蛋白分子由 type-I 轉變爲 type-II 之現象，顯示 evodiamine 可導致 U87-MG cells 進行 autophagy；另外，利用 AnnexinV/Propidium iodide (PI)染劑偵測 apoptosis 百分比，以 evodiamine 處理 48 小時後，apoptosis 百分比達 33.0 ± 3.9 % ($p<0.001$)，推測以 evodiamine 處理 U87-MG，可在初期造成 autophagy，而晚期則誘導細胞進行 apoptosis。

進一步研究其機轉，經由 evodiamine 作用後 1 小時，U87-MG 細胞內鈣離子（$[Ca^{2+}]_i$）濃度會立即明顯的上升。使用內質網 IP3 receptor 的抑制劑 2-APB 可以有效抑制 evodiamine 造成的$[Ca^{2+}]_i$上升達 38%及 evodiamine 誘導的 autophagy 達 45%。此外，以粒線體膜電位專一性染劑 JC-1 偵測粒線體膜電位(mitochondrial membrane potential, $\Delta\Psi m$)，結果顯示粒線體膜電位隨 evodiamine 作用時間增加而有去極化(depolarization)現象，且 MPTP 抑制劑 Cyclosporine A(CsA)，可降低 evodiamin 所誘導的 autophagy，並增加 apoptosis，顯示粒線體傷害參與 evodiamin 所導致的 autophagy。綜合上述實驗結果，本論文推測 evodiamine 可造成 U87-MG 細胞內粒線體膜電位下降，誘發 U87-MG 細胞進行 autophagy 及 apoptosis，其中 $[Ca^{2+}]_i$ 濃度變化是調控 evodiamine 誘導 autophagy 的重要訊息傳遞者。

英文摘要

Malignant gliomas are the primary malignant brain tumors which are difficult to remove completely by surgery. Adjuvant chemotherapy turns out to be one of the

alternative treatments. Autophagy, a type II cell death, has been observed as a highly regulated process of degradation and reusing of intracellular proteins and organelles. Accumulated evidence demonstrated that various anticancer therapies induced autophagy among indicated cancer cell types. Evodiamine, one of the major bioactive compounds isolated and purified from Chinese herbs, Wu-Chu-Yu, has the ability to induce apoptosis in different types of cancers. However, the signaling mechanism of evodiamine-induced autophagy remains elusive. For provide a strategy for development of new chemotherapeutic agents, the specific aim of this study is to investigate the molecular signaling of evodiamine-induced cell death in U87-MG, a human malignant glioma cell line.

We performed MTT assay to examine the effect of evodiamine on the growth ability of U87-MG cells. Results showed that evodiamine significantly inhibited the growth of U87-MG cells ($p<0.001$). As revealed by flow cytometry, we demonstrated that the percentage of autophagy reached a plateau of 42.3 ± 7.5 % ($p<0.001$) after 24 h exposure of 6 μM evodiamine. Using immunoblot assay, we also detected the processing of microtubule-associated protein 1 light chain 3 (LC3), indicating that evodiamine is able to induce autophagy in U87MG cells. A parallel experiment also indicated an apoptosis phenomenon which reached to 33.0 ± 3.9 % ($p<0.001$) after 48 h exposure of 6 μM evodiamine, by using Annexin V/Propidium iodide (PI) double staining . These results indicated that autophagy might play as a prelude to apoptosis in evodiamine-treated U87-MG cells. Moreover, a rapid and marked increasement of intracellular calcium ($[Ca^{2+}]_i$) was detected 1 h after evodiamine-treated U87-MG cells . Pre-treatment of an endoplasmic reticulum IP3 receptor blocker, 2-APB, could prevent both intracellular calcium (38%) and evodiamine -induced autophagy (45%). Using JC-1 staining, we also detected that mitochondrial membrane depolarization . A MPTP blocker, cyclosporine A (CsA) decreased the evodiamine-induced autophagy and increased evodiamine-induced apoptosis, suggesting that evodiamine-induced autophagy was regulated by mitochondrial damage. In conclusion, our results indicated that evodiamine induce both autophagy and apoptosis in U87-MG cells, however, autophagy may ahead from and inhibit the apoptosis and these phenomena are involved with mitochondria membrane depolarization and intracellular calcium.