吳茱萸鹼誘導人類食道癌細胞死亡之作用機轉

The effect and mechanism of evodiamine on inducing cell death of human esophageal cancer cells

中文摘要

根據行政院衛生署民國九十五年公佈台灣地區主要癌症死亡原因，食道癌位居第九位，居男性癌症死因的第六位，五年的存活率低於 10%。食道癌早期常無明顯症狀，因此大部分病患都無法於早期獲得診斷，大約只有 30%之病患在診斷之時，有機會接受手術之治療。所以發展新一代藥劑單獨使用或併用他藥來治療食道癌，有即刻與強烈的需要。

吳茱萸中的成分吳茱萸鹼(evodiamine)是多功能物質，具有降血壓、抗心律不整、刺激內皮細胞和抑制巨噬細胞釋放一氧化氮之作用。抗腫瘤的作用包括誘導細胞凋亡、抑制血管生成的作用及抑制腫瘤細胞的侵入和轉移等等。

本實驗首次將吳茱萸鹼作用於人類食道癌細胞株 CE 81T/VGH 和 TE2。研究結果顯示：隨著時間及劑量之增加，吳茱萸鹼對人類食道癌細胞株 CE 81T/VGH 和 TE2 的生長抑制亦隨之增加，在 72 小時可以高達 80%。在加藥處理後皆可以見到 CE81T/VGH 及 TE2 細胞株具有有絲分裂風暴之形態學特徵，所以我們推測造成 CE 81T/VGH 和 TE2 細胞株死亡的方式主要爲有絲分裂風暴。在免疫螢光染色下，CE 81T/VGH 及 TE2 細胞株在藥物處理後 24 小時皆可以見到因中心體數目增加而形成的多端紡錘體細胞。造成 CE 81T/VGH 及 TE2 細胞株中心體異常增多的原因可能爲 Aurora A 的過度表現。與有絲分裂有關的調節蛋白如 MAD1、Bub R1、Aurora B 及 Survivin 亦隨著吳茱萸鹼作用時間的增長而有不同的表現，所以我們推測紡綞體檢查點蛋白的異常表現是吳茱萸鹼造成 CE81T/VGH 及 TE2 細胞株紡綞體檢查點缺失或異常的主要原因。以流式細胞儀分析發現 CE 81T/VGH 及 TE2 細胞株在藥物處理後 24 小時細胞週期停滯在 G2/M。以西方墨點法分析發現 CE 81T/VGH 和 TE2 細胞株的細胞週期調節蛋白 cyclin B1 及 cdk1 在第一天皆有不正常的表現。

吳茱萸鹼在極低濃度下就有很高的細胞生長抑制的效果，所以具有對抗食道癌細胞之作用，因此有可能作爲食道癌的化學治療藥劑。而且吳茱萸鹼可以讓食道癌細胞週期停滯在 G2/M，具有輻射致敏劑的潛力。因此茱萸鹼有可能成爲新一代治療食道癌的治療藥劑。但是吳茱萸鹼是否可以直接用於治療食道癌病患，則需進一步的研究發展。

英文摘要

Malignant tumor is one of the most common causes of death worldwide and cancer-related mortality is expected to increase considerably. Esophageal cancer, usually is unresectable while diagnosis, has dismal prognosis. Despite aggressive

treatment, the overall 5-year survival rate is less than 10%. Clearly, development of new effective therapy in this malignancy remains the critically important issue in clinical practice.

Evodiamine is a naturally occurring bioactive ingredient of Evodia rutaecarpa (Juss.) Benth. Previous studies on evodiamine showed several biological functions including anti-hypertension, anti-arrhythmia, stimulation on endothelial cells and inhibition on nitric oxide release from macrophages. It has also been demonstrated possessing anti-tumor effects on inducing apoptosis as well as inhibiting tubulin polymerization, angiogenesis, tumor invasion, and metastasis.

This study is the first to examine the anti-tumor effect of evodiamine against human esophageal carcinoma cell lines CE 81T/VGH and TE2. The results show that evodiamine inhibited the growth of these two cell lines, up to 80% inhibition at 72 hours, in a dose- and time-dependent manner. Treatment with evodiamine arrested cell cycle at G2/M phase and induced morphological changes characteristic of mitotic catastrophe in both cell lines. By immunofluorescence staining, centrosome amplification and multipolar spindle were observed. To link these mitosis-related cellular events, the expression of proteins involving chk-cdk1-cyclin B mitotic pathway and spindle checkpoints was assessed simultaneously. Upon evodiamine treatment, cdk1 and cyclin B1 were up-regulated accompanied by a moderate increase in phosphorylation of histone 3, validating an arrest at G2/M. The molecules involving centrosome maturation and spindle checkpoint function, such as Aurora A, Mad1, BubR1, Aurora B and Survivin were up-regulated by evodiamine with a similar time to plateau over 4 – 24 hours followed by degradation.

In conclusion, evodiamine possesses growth inhibitory activity against human esophageal cancer cells. The major mode of cell death induced by evodiamine is mitotic catastrophe accompanied by G2/M arrest and centrosome amplification. The putative mechanisms of action might be those mediating centrosome maturation, amplification and spindle checkpoint function. It indicates that evodiamine might be a potent and novel therapeutic agent against esophageal cancer and may have a role in radiation sensitization. Further in vivo studies including anti-tumor effect and toxicity are warranted.