2-(Naphthalen-2-ylmethylsulfanyl)-5,5-diphenyl-1,5-dihydro-imidazol-4-one（SDil-N10）對於人類血管內皮細胞的生長抑制作用

The anti-proliferation effect of 2-(Naphthalen-2-ylmethylsulfanyl)-5,5-diphenyl-1,5-dihydro-imidazol-4-one（SDil-N10）in human vascular endothelial cells

中文摘要

本篇論文的主旨，在研究化學合成物質 SDil-N10【2-(Naphthalen-2-ylmethylsulfanyl)-5,5-diphenyl-1,5-dihydro-imidazol-4-one】對於人類臍靜脈內皮細胞 (HUVEC) 的影響，並探討其中作用的機制。我們實驗結果顯示 SDil-N10 可抑制 HUVEC 細胞的生長，且其抑制作用與藥物濃度及處理時間呈正向相關。[3H] Thymidine incorporation 的實驗結果顯示，SDil-N10 可抑制 HUVEC 細胞的 DNA 合成作用，但對人類纖維母細胞 Fibroblast 的生長影響則相對小很多。西方墨點法 (Western blot analysis) 的實驗結果觀察到 SDil-N10 處理細胞 24 小時，其和細胞週期停滯有關的 p21 以及 p27 蛋白表現量高於對照組，同時 Cyclin A 蛋白的表現量有顯著下降，而 p53、CDK2、CDK4、Cyclin E、Cyclin D1、Cyclin D3 蛋白的表現量則無明顯變化。免疫沉澱法與蛋白激酶活性測定實驗結果發現以 SDil-N10 處理後，能增加 p21 與 CDK2 的結合量，並降低 CDK2 的激酶活性。實驗也發現 SDil-N10 能夠抑制 vascular endothelial growth factor (VEGF) 所誘導之內皮細胞增生的現象。2D-Matrigel 微小管腔形成以及 Rat Aorta 微小管腔形成 (tube formation) 實驗結果發現給予 SDil-N10 能夠抑制內皮細胞微小管腔形成的情況。我們認爲 SDil-N10 會干擾內皮細胞的細胞週期進行，因而減少細胞增生，其作用主要透過抑制 CDKs 活性的途徑。因此，SDil-N10 有機會可以成爲抗血管增生的藥物。

英文摘要

The aim of this study was to examine the anti-proliferation effect of 2-(Naphthalen-2-ylmethylsulfanyl)-5,5-diphenyl-1,5-dihydro- imidazol-4-one (SDil-N10), an analogue of antiepileptic drug phenytoin(5,5-diphenylhydantoin, DPT), on human umbilical vein endothelial cells (HUVEC) and its possible molecular mechanism underlying. SDil-N10 at a range of concentrations (10-50 uM) dose- and time-dependently inhibited DNA synthesis and decreased cell number in cultured HUVEC, but less effect in human fibroblasts. [3H] Thymidine incorporation assay demonstrated that treatment of HUVEC with SDil-N10 arrested the cell at the G0/G1 phase of the cell cycle. Western blot analysis revealed that the protein levels of p21 and p27 increased and cyclin A decreased after SDil-N10 treatment. In contrast, the protein levels of p53, cyclin D1, D3 and E, cyclin-dependent kinase (CDK2, and CDK4) in HUVEC were not changed significantly after SDil-N10 treatment. Immunoprecipitation showed that the formation of the CDK2-p21 complex, but not the CDK4-p21, CDK2-p27 and CDK4-p27 complex, was increased in the SDil-N10-treated HUVEC. Kinase assay further demonstrated that CDK2, but not CDK4, kinase activity was decreased in the SDil-N10-treated HUVEC. SDil-N10 also inhibited vascular endothelial growth factor (VEGF) induced endothelial cells proliferation. 2D-Matrigel and rat aorta tube formation assays further showed that SDil-N10 inhibited HUVEC tube formation. Taken together, these data suggest that SDil-N10 inhibits HUVEC proliferation by increasing the level of p21 protein, which in turn inhibits CDK2 kinase activity, and finally interrupts the cell cycle. The findings from the present study suggest that SDil-N10 might have the potential to inhibit the occurrence of angiogenesis