# 黃芩?元活化 Akt 及低氧誘導因子活性之神經保護作用研究

# Study of baicalein neuroprotection via enhancement of Akt and hypoxia-inducible factor-1α activities in cortical neurons


**中文摘要**

民國 94 及 95 年，連續兩年腦血管疾病與事故傷害分居十大主要死因之第二與第五名；而中樞神經因腦血管疾病或事故傷害受損後，興奮性胺基酸麩胺酸(glutamate)將大量釋放，繼而更進一步造成續發性的神經損傷。本實驗室曾發表中藥黃芩(Scutellaria baicalensis Georg)萃取物:Baicalein 於興奮性胺基酸麩胺酸造成之神經興奮毒性中具有神經保護效果(neuroprotection)；然而，其分子機制仍待探討。本論文主旨即研究所提供神經保護功能之分子機制。實驗以成長 17 至 18 天大白鼠(Sprague Dawley, SD)胚胎之大腦皮質神經元(cerebral cortical neurons)體外培養至神經成熟後爲研究系統。給予麩胺酸及 N-methyl-D-aspartate (NMDA)模擬神經損傷。Baicalein、麩胺酸及 N-methyl-D-aspartate (NMDA) 共同投予下，確實能夠降低麩胺酸與 NMDA 所增加之乳酸脫氫酵素釋放[lactate dehydrogenase (LDH) release assay; 細胞死亡程度測試]。於細胞訊息傳遞中，被磷酸化的 Akt，能活化其下游之促進細胞存活因子(例：HIF-1α)而引導細胞趨向存活。而不論是否有神經損傷的情況下，都能提高 Akt 之磷酸化 (phosphorylation)；推測其進而穩定 hypoxia inducible factor-1 alpha (HIF-1α)且使 HIF-1α成爲更具有進核(nuclear translocation)能力之轉錄因子(transcription factor)。我們進而發現 baicalein 能提高以 HIF-1α爲轉錄因子的 HRE (Hypoxyia Responsive Elements) 驅動之 reporter 基因表現。染色質免疫沈澱(Chromatin Immunoprecipitation)實驗進一步證實了 baicalein 能夠促使 HIF-1α結合至 erythropoietin (EPO)與 vascular endothelial growth factor (VEGF) 驅動子(promoter)基因序列上的 HRE；而根據此結果，進行反轉錄聚合酵素鏈鎖反應(reverse transcriptase PCR) 證實 baicalein 會增加 HIF-1α所調控之細胞保護基因(EPO 及 VEGF)的 mRNA 表現。而 EPO 也於其他報導中呈現神經細胞保護效果。由於 baicalein 引發這些神經保護基因表現需要 8-12 小時，我們進一步證實了其神經保護作用必須以在興奮性胺基酸引發神經損傷前 8-12 小時投予才會有效，且此保護性可被 PI3K inhibitor 所抑制。總結上述，本論文進一步闡明了 baicalein 神經保護作用的分子機制之一，即可能來自於其能夠活化 Akt 及 HIF-1α，進而引發神經保護基因 EPO 之表現。

**英文摘要**

Glutamate-induced excitotoxicity is believed be involved in several

neurodegenerative diseases, including stroke, brain/head injury, Alzheimer''s disease and Parkinson''s disease. Our previous study has demonstrated that baicalein, one of the flavonoids extracted from Scutellaria baicalensis Georgi, protects primary cortical neurons from glutamate/NMDA induced cell death. In this study, we further revealed that baicalein treatment enhanced phosphatidylinositol 3-kinase (PI3K)-mediated Akt phosphorylation, which is known to mediate survival signal in cells under detrimental insults. Furthermore, 24 h baicalein treatment dose-dependently increased the expression of luciferase reporter gene driven by human erythropoietin gene promoter that contains hypoxia-responsive element (HRE) for the binding of hypoxia-inducible factor 1α (HIF-1α); and this increase was reversed by PI3K inhibitor LY294002. Furthermore, the nuclear HIF-1α was increased by baicalein after 30 min and 60 min treatment, but this effect was not reversed by PI3K inhibitor. Chromatin immunoprecipitation (ChIP) shows that HIF-1α binds to HRE on erythropoietin (EPO) and vascular endothelial growth factor (VEGF) enhancer. In this regard, baicalein was found increasing of the EPO and VEGF gene expression by RT-PCR analysis. Consequently, the mitochondrial dysfunction resulted from glutamate and NMDA was significantly reversed baicalein pretreatment (12 or 8 hour), and this reversion was blocked by PI3K inhibitor. In conclusion, our results suggest that baicalein may activate Akt and HIF-1α, and might in turn give rise to more HIF-1α available for transactivating neuroprotective genes, EPO and VEGF, expression.