Baicalein 影響小鼠黑色素瘤細胞株產生 ROS 之作用機轉探討

Mechanism of the Baicalein induced Reactive Oxygen Species Formation in Mouse B16F10 Melanoma Cell

中文摘要

漢方草藥黃芩根部萃取物之黃芩素(baicalein; 5,6,7,-trihydroflavone)爲一種類黃酮物質，被認爲具有抗發炎抗癌的特性。在過去的文獻中說明 baicalein 在血小板會透過 12-lipoxygenase 去引發氫氧自由基 (hydroxyl radical)和透過 PGHS peroxidase 去引發半醌自由基 (semiquinone radical)的生成。本次研究我們利用電子順磁共振的技術，發現 baicalein 可以在 B16F10 小鼠黑色素瘤細胞株以濃度相關性方式去引發 hydroxyl radical和 superoxide 訊號產生。利用 phospholipase A2 (cPLA2) 抑制劑，AACOCF3 和 quinacrine 和高濃度的 arachidonic acid 可以有效的減弱 hydroxyl radical訊號的強度，因此認爲和 AA 代謝的酵素有關。而利用 mannitol和 catalase 這兩種 ROS 的清除劑，也可以有效的抑制氫氧自由基的生成。 (-)-Epicatechin 和 (-)-epicatechin gallate 兩者屬於 benzene-1,3-diol類的 flavonoids 也同樣被作爲 12-LOX 的抑制劑，但是在 ESR study 卻無法在 B16F10 小鼠黑色素瘤細胞偵測到任何的 hydroxyl radical訊號。利用 siRNA 方式抑制 12-LOX 蛋白表現可以減弱高濃度的 baicalein 對細胞所刺激生成的 hydroxyl radical。另外在 western blotting 實驗中我們發現 baicalein 可以刺激細胞內 active caspase-3 蛋白的活性，但是卻無法有效的抑制 12-LOX 蛋白的表現。在 MTT 實驗中 baicalein 可以讓細胞生長受到抑制但是在投與 ROS 清除劑後卻可以讓細胞存活率顯著的回復。因此在眾多實驗數據顯示中我們認爲 baicalein 刺激 B16F10 小鼠黑色素瘤細胞生成的 superoxide 會經由 superoxide dismutase (SOD) 和 fenton reaction 去生成對細胞具有傷害性的 hydroxyl radical。Baicalein 可以做爲一個強力的電子提供者去和脂質過氧化自由基的中間產物透過 12-LOX 去做 co-oxidation 動作進而造成細胞產生 hyroxyl radical。同時 baicalein 也可以刺激 caspase-3 的活化，這些可能就是造成 baicalein 造成細胞存活率下降和細胞凋亡的重要證據

英文摘要

Baicalein (5,6,7,-trihydroflavone) as flavonoid originated from the root of Chinese medical herd Scutellaria baicalensis, has been shown to exert anti-inflammatory and anti-cancer effects. In previous study demonstrated that baicalein induced hydroxyl radical formation via 12-lipoxygenase (12-LOX) and induced semiquinone radical formation via PGHS-peroxidase in human platelets. Here we found that baicalein also induces hydroxyl radical and superoxide formation in B16F10 melanoma cell line in

dose dependent manner by using electron spin resonance (ESR) technique. Two phospholipase A2 inhibitors (cPLA2), AACOCF3 and quinacrine and a high concentration of arachidonic acid (AA) attenuates the intensity of hydroxyl radical signal indicating that baicalein induced hydroxyl radical formation is associated with AA metabolite enzymes. Two ROS scavengers, mannitol and catalase, also significantly inhibit the intensity of hydroxyl radical signal in ESR study. (-)-Epicatechin and (-)-epicatechin gallate which are flavonoids contain benzene-1,3-diol structure are also used as 12-LOX inhibitors, but they showed rarely detectable signal of hydroxyl radical after incubated with B16F10 melanoma cell suspension in ESR study. Cell transfected with 12-LOX siRNA could attenuate hydroxyl radical signal which induced by high concentration of baicalein. According to western blotting results, we found that baicalein had the activated effect in caspase-3 activity but had no effect in 12-LOX expression. In MTT assay, we also found that baicalein caused reduction of cellular viability and were reverse by addition of ROS scavengers. Present data indicate that superoxide induced by baicalein could promptly convert to hydroxyl radical through superoxide dismutase (SOD) and fenton reaction in B16F10 melanoma suspension. Baicalein pose as a strong electron donor which co-oxidation with fatty acid peroxy radical intermediate through 12-LOX to induce hydroxyl radical formation. These may be the evidence that baicalein caused cell growth retardation and cell apoptosis.