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• 中文關鍵字	細胞凋亡；白血病細胞；漢黃芩素；卡斯帕西斯；核酸內切酵素？？
• 英文關鍵字	Apoptosis；Leukemia cell；Wogonin；Caspase；Endonuclease；Fisetin
• 中文摘要	本計劃探討 wogonin 與 fisein 誘導血癌細胞 HL-60 凋亡之機轉。研究結果證實 wogonin 與 fisein 能有效誘導血癌細胞 HL-60 走向細胞凋亡，其過程會伴隨著 DNA 階梯斷裂、凋亡小體之出現與細胞週期不正常變化。我們同時也發現 caspase 3 (not caspase 1)被活化、PARP 被切斷與 endonuclease 活化。此 endonuclease 爲 Ca+2 depedent 且其適合之活性 pH 值爲 6.5 。以 caspase3 抑制劑處理能抑制 wogonin 與 fisein 誘導血癌細胞 HL-60 凋亡與 endonuclease 活化。此結果顯示 caspase 3 與 endonuclease 在 wogonin 與 fisein 作用機轉中很重要。在探討 Bcl-2 family proteins 表現方面，wogonin 與 fisein 誘導 Bax 蛋白質產生同時抑制 MCl-1 蛋白質表現。NAC 或 catalase 能有效抑制 H2O2 誘導細胞凋亡，然而無法有效抑制 wogonin 與 fisein 誘導之細胞凋亡，推測自由基產生不包含在其作用機轉。以 DCHF-DA 偵測細胞內 peroxide 量之變化，結果指出 wogonin 與 fisein 能明顯減少細胞內之 peroxide 量。本結果證實 wogonin 與 fisein 能經由活化 caspase 3 與 endonuclease 而殺死血癌細胞，且此活性與降低細胞內 ROS 量有相關。（accepted byBiochemical Pharmacology ）
• 英文摘要	Seven structurally related flavonoids including luteolin, nobiletin, wogonin, baicalein, apigenin, myricetin and fisetin were used to study their biological activities on the human leukemia cell line, HL-60. On MTT assay, wogonin, baicalein, apigenin, myricetin and fisetin showed obvious cytotoxic effects on HL-60 cells, with wogonin and fisetin being the most-potent apoptotic inducers among them. The cytotoxic effects of wogonin and fisetin were accompanied by the dose- and time-dependent appearance of characteristics of apoptosis including DNA fragmentation, apoptotic bodies and the sub-G1 ratio. Treatment with an apoptosis-inducing concentration of wogonin or fisetin causes rapid and transient induction of

caspase 3/CPP32 activity, but not caspase 1 activity. Further, cleavage of poly(ADP-ribose) polymerase (PARP) and decrease of pro-caspase 3 protein were detected in wogonin and fisetin treated HL-60 cells. An increase in the pro-apoptotic protein, bax, and a decrease in the anti-apoptotic protein, Mcl-1, were detected in fisetin- and wogonin- treated HL-60 cells. However, Bcl-2, Bcl-XL, and Bad all remained unchanged in wogonin- and fisetin-treated HL-60 cells. In vitro chromatin digestion revealed that endonuclease activity was profoundly enhanced in wogonin- and fisetin-treated HL-60 cells, and the addition of EDTA or EGTA into the reaction blocked endonuclease activation and at an optimum pH of 7.5. The caspase 3 inhibitor, Ac-DEVD-CHO, but not the caspase 1 inhibitor, Ac-YVAD-CHO, attenuated wogonin- and fisetin- induced DNA ladders, PARP cleavage, and endonuclease activation. Pre-treatment of HL-60 cells with N-acetyl-cysteine or catalase efficiently inhibited H2O2 (200 mM)-induced apoptosis, but showed no inhibitory effect on wogonin- and fisetin-induced DNA ladders, caspase 3 activation, or bax protein induction. Decrease in endogenous ROS production was detected in wogonin- and fisetin-treated HL-60 cells by DCHF-DA assay. In conclusion, our experiments indicate that a decrease in intracellular peroxide level was involved in wogonin- and fisetin-induced apoptosis; activation of caspase 3 and endonuclease, induction of bax protein and suppression of Mcl-1 protein were detected in the process.