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一、中文摘要

黃芩甘 (Baicalin) 為唇型科 (Labiatae) 植物「黃芩」(Scutellaria baicalensis GEORGI) 中所含天然的類黃酮成份。根據報告漢黃芩素對肝炎患者血清中 Xanthine oxidase 具有抑制作用(1)。臨床上黃芩對急性黃膽型肝炎，急性無膽型肝炎及慢性 B 型肝炎有一定療效 (2)。從我們過去所作的研究和過去的報告 (3, 4)顯示採用有機過氧化劑 t-BHP (Tert-butyl hydroperoxide)誘導肝細胞脂質過氧化的氧化性傷害 (oxidative stress)已建立了良好的生藥活性成份之研究模式。而且在先前的研究計劃中我們已針對 Baicalein 進行抗癌機轉的探討，並已發現 Baicalein 有及好的抗癌作用 (5)，因此我們探討 Baicalein 存在下，經 TPA 處理之 CD-1 mouse 的上皮組織中，對 TPA 所誘發的 PKC 量及上述訊息傳遞蛋白表現量的影響，藉以了解 Baicalein 抗癌之分子機制是經由何項途徑。以西方墨點吸漬法測定蛋白之表現，結果發現預先處理 0.4 mM Baicalein 會引起 PKCα, c–Raf, MEK-1, ERK1/ERK2, c-Jun 和 c-Myc 蛋白的表現受到抑制，其抑制百分比分別為 74%, 4%, 24%, 32%, 62%, 55%, 5%, -16%, 30%。而高劑量 1.0 mM Baicalein 則有更明顯的抑制，其抑制百分比分別為 96%, 71%, 47%, 73%, 91%, 91%, 27%, 42%, 83%。綜合這些結果及先前的研究顯示，Baicalein 抑制 TPA 引起腫瘤促進作用的機轉，可能是經由阻斷 TPA 所引起的訊息傳遞蛋白表現來完成。

Abstract

Baicalein, a major component present in the root of *Scutellaria baicalensis* GEORGI, has been reported previously to inhibit the cell growth rate on human hepatoma cell lines (PLC/PRF-/5 and Hep-G2), human liver cell and human pancreatic cancer line. Furthermore, baicalein was found to suppress proliferation not only on vascular smooth muscle cells but also on T-lymphoid leukemia cells through reducing the protein tyrosine kinase activity and protein kinase C activity induced by PMA. In our previous study, it was found that baicalein possessed antioxidative ability and antitumor promotion induced by TPA in CD-1 mice skin. In this study, to clarify the working mechanism of baicalein, we investigate the upstream signaling pathways which lead to the gene expression during the baicalein-protected tumor promotion in mouse skin. We examine the expression of PKCα, c-Raf, MEK-1, ERK1/ERK2, c-Fos, c-Jun and c-Myc. The results showed that mice skin pretreated with three different concentrations of baicalein (0.4 mM, 0.8 mM and 1.0 mM) result in the reduction of the expression of PKCα, c–Raf, MEK-1, ERK1/ERK2, c-Jun and c-Myc by a dose-dependent

manner. More mitogen-activated proteins and early gene products will be investigated. In conclusion, our results show that the inhibitory effect of baicalein on tumor promotion may involve the suppression of the MAPKs.

二、緣由與討論

惡性腫瘤是國人十大死因之一，且目前已高居首位 (6)，根據統計平均每四人中有一人得過腫瘤，可以說已達到人人談癌色變的程度；造成癌症的原因尚爲完全清楚，一般來說肇因於下列因子: (1) 輻射線曝露 (2) 環境污染 (3) 化學致癌物 (4) 病毒感染 (5) 個人生活習慣；可是有一點可以確定的是人類因長期慢性的感染或發炎則爲發生炎症的重要因子，由於發炎組織中常產生大量的活性氧自由基 (Reactive oxygen species)，如 O2-，RO.，H2O2 等，而氧自由基爲細胞癌化起始其的重要因素 (7)，氧自由基具有獨字癌化細胞的能力，由於人體餒所產生的氧自由基與抗氧化物系統間之平衡，及是身體受氧自由基危害程度的決定因素，一旦人體內此一平衡受到破壞則導致組織器官產生病理變化而形成腫瘤演變成癌症。環顧目前醫學界治療癌症，雖有外科手術，放射線照射治療或抗代謝藥物以阻斷癌細胞快速增長，但因其往往也傷害到正常細胞，或療效不佳或併發許多副作用如貧血、脫髮、惡心、嘔吐...等，可是天然物中有不少抗癌生藥，若能由其單離出之純的活性化學成分，採用現代生化藥理學的科學方法加以探討研究，以尋求一有效抗癌藥物，俾供臨床使用，實爲刻不容緩之事。

黃芩 (Scutellaria baicalensis GEOEGI)爲唇型科 (Labiatae)植物的乾燥根，民間常用爲清熱利濕之中草藥，其功能清熱燥濕，瀉火解毒，利小便 (1)。根據研究指出黃芩具有清熱解毒，消除炎症，擴張血管，加速膽汁排泄，從而幫助消化，促進食慾，同時，還可擴張血管，改善肝內循環 (8, 9)；黃芩甘對肝癌患者血清中 Xanthine oxidase 具有抑制作用 (1)。臨床上黃芩能治急性黃膽性、急性無膽性肝炎及慢性 B 型肝炎(2)。Baicalein 爲由黃芩中分離出三種類黃酮，根據報告一般類黃酮成份多具有抗炎、抗過敏及抗氧化作用(10, 11)。

先前的研究利用癌起始劑 Benzo[a]pyrene (B[a]P)及癌促進劑 12-O-tetradecanoylphorbol-13-acetate (TPA)使小白鼠皮膚引致腫瘤，再以預先處理 Baicalein 的小白鼠相互比較得知 Baicalein 可抑癌。也對 TPA 引起之發炎反應有抑制情形，包括老鼠耳朵的水腫、上皮的 hyperplasia 及發炎、ODC 活性、H_2O_2 形成與 MPO 活性。最近我們已有發表論文指出 Baicalein 可以抑制 TPA 所引起的小白鼠皮膚癌促進作用，而 Baicalein 的抑制機轉則至今未見報告。在從前的報告中指出 PKC activity 可在 TPA 的刺激下增強並進行下游訊息的調節，包括活化 mitogen-activated protein kinases (MAPKs)，活化後之 MAPKs 會藉由其磷酸化而調節基因表現的相關蛋白激媒，需要 TPA 刺激而表現之 transcriptional gene 包括 c-jun 及 c-fos，此基因係經由 TPA 刺激 PKC 活化及 MAPKs 之活化而被激活(12-20)。本計劃之主要目的在於探討 Baicalein 抑制 TPA 引起之老鼠上皮細胞的癌促進作用的機轉，而在我們的結果中顯示 Baicalein 的抑制作用乃在於經由抑制 PKC 所引起的 MAPKs 活化作用，並經由此抑制作用而抑制了位於細胞核的原致癌基因，藉此使得 TPA 的促癌作用被抑制。

三、結果與討論

分別以 TPA 及不同濃度 baicalein 處理在老鼠上皮，每天 2 次，連續五天後取下上皮之蛋白質以 Western Blot 的方法偵測在 baicalein 對 TPA 所引起之 MAPK pathway 活化之抑制作用，分別測定的是 Raf-1, MEK-1, ERK-1, ERK-2, TPA 在 Raf-1, MEK-1, ERK-1, ERK-2 分別被 induction，在預先處理 baicalein 的組別中，依三種不同濃度分別對 Raf-1, MEK-1, ERK-1, ERK-2 產生抑制作用(Fig.1)。

在 protooncogenes 表現上，則同樣以 Western Blot 來觀察蛋白表現的量，包括 c-Fos, c-Jun 及 c-Myc 的表現，TPA 在 c-Fos, c-Jun 及 c-Myc 分別被 induction，在預先處理 baicalein 的組別中，依三種不同濃度分別對 c-Fos, c-Jun 及 c-Myc 產生抑制作用 (Fig.2)。

由於在 TPA 處理過的皮膚上會有 PKC 被誘發而表現的現象，因此我們仍以上述之方法來測定 PKC 的表現，另外在磷酸化酪氨酸蛋白質 (phosphotyrosine protein, Py) 的測定，則可以藉此觀察 baicalein 對 TPA 所引起的蛋白質磷酸化的抑制情形，在 TPA 處理的組別中 PKC-α 及 Py 分別被 induction，而在預先處理 baicalein 的組別中，依三種不同濃度分別對 PKC-α 及 Py 產生抑制作用 (Fig.3)。

由此結果可以得知在老鼠上皮給予 TPA 後會使得過氧化物急速增加，此種增加則誘使蛋白質進行磷酸化而啓動 MAP kinases cascade 進行訊息之傳遞，最後活化細胞核內之 protooncogenes 而引發癌化的進行。baicalein baicalein 在此過程中應是因爲抑制了過氧化物之產生進而使下游訊息傳遞受到抑制而達成保護細胞免於癌化的危機。

四、計劃成果自評

從天然物中發現新的 compound 來做為癌症治療藥物，並研究其對癌細胞作用之機制，在未來癌症醫學上應是重要工作之一。

本計劃之目標爲找出 Baicalein 抑癌作用之機轉，研究成果中顯示 Baicalein 可能係經由抑制了過氧化物引發之訊息傳遞，使得下游之 protooncogenes 免於被誘發，藉此保護個體不會進行癌化的過程。至於是否有其它相關途徑包含於其中，值得進一步探討及研究。

五、參考文獻

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Table 1 ***Inhibitory effect of topical application of baicalein on TPA-induced edema of mouse ears***

Group	Treatment[a]	Weight/punch/mg	inhibition / %[b]
1	Acetone	7.50 ± 3.14	-
2	TPA	16.62 ± 2.70	-
3	Baicalein (0.08 nmol)+TPA	13.17 ± 1.85**	38
4	Baicalein (0.16 nmol)+TPA	12.37 ± 2.21**	47
5	Baicalein (0.2 nmol)+TPA	11.75 ± 2.05**	53

[a] Mouse ears were treated with acetone (20 μl), TPA (0.5 nmol) in acetone (20 μl) or TPA (0.5 nmol) together with baicalein in acetone (20 μl). Five hours later, the animals were killed and ear punches (diameter 6 mm) were weighed. The data represent the mean ± SD from 6 mice/group.

**$P < 0.001$. Statistically different from TPA group, *t*-test.

[b] Percentage of inhibition (%) = [Group 2 - Group 3 (or 4, or 5)] / [Group 2 - Group 1] X 100%.

Table 2 ***Inhibitory effect of topical application of baicalein on the TPA-induced morphological changes in epidermis***

Group	Treatment[a]	No. of epidermal layers	Epidermal thickness (μm)	Leukocyte infiltration[b]	Intercellular edema[c]
1	Acetone	2-3	15.63±5.12	0	0
2	TPA	4-7	70.00±16.58	++	+
3	TPA + Baicalein (0.08 nmol)	3-6	53.75±17.85*	++	0
4	TPA + Baicalein (0.16 nmol)	3-5	48.43±15.14**	++	0
5	TPA + Baicalein (0.2 nmol)	2-4	45.75±8.47**	+	0

[a] Mice were treated topically with acetone (200 μl), TPA (5 nmol) in acetone (200 μl), or TPA (5 nmol) together with baicalein in acetone (200 μl) twice a day for four days. The animals were killed 18 h after the last dose and skins were processed for histological tests. Data are expressed as the mean ± SD from 6 mice/group.

* $P < 0.01$, ** $P < 0.001$. Statistically different from TPA group, *t*-test.

[b] Leukocyte infiltration that was slight (+) or serve (++) was characterized by diffuse infiltration of mononuclear infiltratory cells in the dermis when compared with the acetone controls.

[c] Intracellular edema was scored as present (+) or absent (0).

Fig.1 Western blotting examination of the effect of Baicalein on TPA-induced the expression of MAP cascade kinases

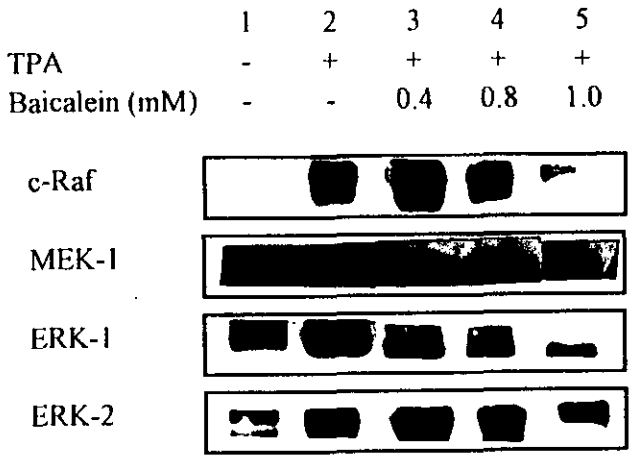

Fig.2 Western blotting examination of the effect of Baicalein on TPA-induced nuclear-protocogenes protein

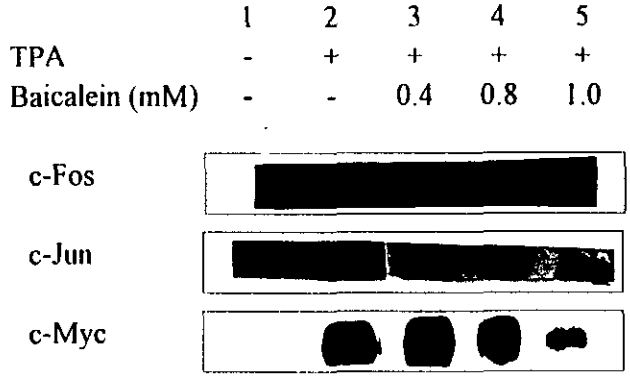

Fig.3 Western blotting examination of the effect of Baicalein on TPA-induced the expression of PKC-α and phosphotyrosine protein