行政院國家科學委員會補助專題研究計畫成果報告

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※　　抗氧化物 Silymarin 對免疫細胞的作用　　※

※　The Effect of an Antioxidant, Silymarin, on Immune Cells　※

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計畫主持人：林大盛

共同主持人：

計畫參與人員：

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抗氧化物 Silymarin 對免疫細胞的作用

The Effect of an Antioxidant, Silymarin, on Immune Cells

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一、中文摘要

Milk thistle (Silybum marianum) 有效的萃取物爲 silymarin，乃民眾最常用來治療肝病之草藥。一些證據指出 silymarin 作用類似維生素 E，亦爲 membrane-active antioxidant，可保護細胞膜因 H_2O_2 及其他自由基的傷害，亦有提升淋巴細胞及紅血球之 superoxide dismutase 活性，因而認爲 silymarin 之抗氧化作用爲其肝臟保護能力之一重要因子。另外，一些研究也指出 silymarin 可影響淋巴細胞的活化，增強 lectin 對淋巴細胞的作用，提示了 silymarin 亦可藉由影響體內免疫細胞反應，進而達到肝臟保護效果。雖然 silymarin 對肝臟之療效尚待進一步臨床實驗證實，但如上所述，在世界各地 silymarin 已普遍被用於治療人及動物肝相關的疾病，故有必要了解其對體內免疫系統之影響。由於 silymarin 各種活性類似維生素 E，而維生素 E 已被證實並非只是單純的抗氧化物，亦爲免疫調控物質，故本研究之目的在探討 silymarin 是否亦爲免疫調控物質。即探討 silymarin 影響巨噬細胞分泌 IL-1 及 tumorcidal activity，刺激 lymphocyte proliferation，以及影響 Th1/Th2 型免疫反應。

結果發現 Silymarin 本身對淋巴細胞有直接刺激效果，在同時有 PHA 存在下刺激效果會更好。同時，silymarin 有助於受刺激細胞 Th1 及 Th2 型之反應，但前者效果比後者好。Silymarin 在 LPS 刺激下可增強巨噬細胞活性。

關鍵詞：silymarin，免疫調控，細胞免疫，Th1/Th2 型細胞素

Abstract

Silymarin, the active extract of milk thistle (Silybum marianum), has been used for the treatment of liver diseases for centuries. It is evident that biological activities of silymarin are similar to vitamin E being membrane-active antioxidant. Silymarin can protect cell membrane damage done by H_2O_2 and other free radicals and also can enhance the superoxide dismutase activity of lymphocytes and erythrocytes. Therefore, it is believed that antioxidant activity is an important ability of silymarin for liver protection. In addition, some studies indicate that silymarin can influence lymphocyte activation and enhance lectin-induced lymphoblast transformations. Thus, it is suggested that silymarin may also offer liver protection ability by influencing immune cell responses. Although the

efficiency of silymarin for the therapy of liver diseases still needs verification by further human clinical trials, as described above silymarin has already been used widely for the therapy of human and animal liver diseases. Therefore, it is necessary to understand the effects of silymain on immune system in the body. Because biological activities of silymarin are similar to vitamin E, which has been proved to be an immunomodulator, the purpose of this study will investigate if silymarin also an immunomodulator as well. The following items will be examined for the effects of silymarin on immunity: IL-1 secretion and tumorcidal activity of macrophages, stimulation of lymphocyte proliferation, and influence of Th1/Th2 type immune responses.

It was found that silymarin could directly stimulate lymphocyte proliferation. This effect would be significantly enhanced when PHA was also present. At the same time, silymarin promoted both Th1 and Th2 responses. However, the former was enhanced greater than the latter. Silymarin plus LPS could enhance both IL-1 secretion and tumorcidal activity of macrophages.

Keywords: silymarin, immunomodulation, cell-mediated immunity, Th1-/Th2-type cytokines

二、緣由與目的

Milk thistle (Silybum marianum) 之衍生物作爲草藥療病已有 2000 年久之歷史。目前 milk thistle 萃取物 (silymarin) 爲民眾最常用來治療病毒性肝炎及其他肝病之草藥 (Brevoort 1996)。Milk thistle 有效的萃取物爲 silymarin (爲 flavonoligans，silydianin，silychristine 及 silybin 之混合)，其中以 silybin 最具生物活性 (Flora et al., 1997)。一些證據指出 silymarin 作用類似維生素 E，對細胞沒毒性 (Meroni et al., 1988)，亦爲 membrane-active antioxidant (Hermansky et al., 1991)，可保護細胞膜因 H_2O_2 (Anderson et al., 1994; Halim et al., 1997) 及其他自由基如 hydroxyl anions (OH^-) 和 hypochlorous acid (HOCL) (Feher et al., 1990; Mira et al., 1994) 的傷害。除了最常用來治療病毒性肝炎外，可保護及治療因毒物 (Paulova et al., 1990) 或 ischemia (Wu et al., 1993) 導致的肝臟傷害。亦有提升淋巴細胞及紅血球之 superoxide dismutase 表現與活性 (Feher et al., 1998; Muzes et al., 1991; Lang et al., 1993)，因而增強抗氧化能力。證據指出，silymarin 之抗氧化能力和肝臟維護能力有關 (Lang et al., 1990a,b; Rauen & de Groot, 1998)。Silymarin 亦被使用於家畜及家禽。silymarin 可防止因 sawfly (Arge pullata) 導致的綿羊肝中毒 (Thamsborg et al. 1996)，也可阻斷狗肝藏對毒物之吸收 (Faulstich et al., 1980)，具有保護及治療狗 (Vogel et al., 1984; Paulova et al., 1990) 肝中毒之效用。同時，silymarin 亦當做一種抗氧化劑使用於狗隻 (Konya et al., 1990; 1993)。Silymarin 可影響淋巴細胞的活化 (Meroni et al., 1988)，增強 lectin 對淋巴細胞的作用 (Lang et al., 1990a)，減少 $CD8^+$ 細胞比率 (Lang et al. 1990a)，降低 natural killer (NK) cells 活性 (Deak et al., 1990)，以及提升 neutrophils 之活動力 (Kalmar et al., 1990)，提示了 silymarin 亦可藉由影響體內免疫細胞反應，進而達到肝臟保護效果。另外，silymarin 因具有穩定 mast cell 細胞膜之特性，所以也有抗過敏作用

(Miadonna et al., 1987)。由於 silymarin 之各種活性類似維生素 E，而維生素 E 已被證實並非只是單純的抗氧化物，亦爲免疫調控物質 (immunomodulator) (Oonishi et al., 1995; Traber & Packer, 1995; Sakai & Moriguchi, 1997)。吾人更進一步證實維生素 E 可刺激淋巴球並提升其 Th1 cytokines 之釋放，同時也可促進 macrophages 之 tumorcidal activity。故本研究之目的在探討 silymarin 是否爲免疫調控物質。即探討 silymarin 影響巨噬細胞分泌 IL-1 及 tumorcidal activity，刺激 lymphocyte proliferation，以及影響 Th1/Th2 型免疫反應。

三、結果與討論

使用 MTT 來衡量細胞被刺激進而活化、分裂的程度。MTT 是種 tetrazolium salt，活細胞的粒線體可將 MTT 轉變成紫色結晶，4 小時後加入 sodium dodecyl sulfate 及 HCl 之 solvent 以溶解紫色結晶，置於 37^{o}C incubator。隔日以 ELISA reader (wavelength ： 570 nm/690 nm) 讀其 absorbance (optical density) 值。活細胞的數目和液體顏色強度成正比關係。Silymarin (20μg/ml) 有直接刺激淋巴球 (2.5 x 10^5/well) 增殖能力，但在和 PHA 共同存在下對淋巴球刺激效果比只有 PHA 好。

進一步想了解在有無 silymarin 的情形下，以 ELISPOT 法偵測細胞受 PHA 的刺激是否會影響 Th1 cytokine 及 Th2 cytokine 分泌細胞的增殖。使用孔內有 nitrocellulose base 之 96 孔盤 (Milititer HA; Millipore)。加入 50 μl 之抗 Th1-cytokine (IL-2) 或抗 Th2-cytokine (IL-4) 之 monoclonal antibody [10 μg/ml in phosphate- buffered saline (PBS), pH 7.4]，4^{o}C，overnight。以無菌 PBS 洗二次，加入 200μl 含有 fetal calf serum 之 RPMI 1640 culture medium，置於室溫 1 hr 之後，然後加入待測之細胞 (1 x 10^5/100μl/well)。培養 20 hr 後，以含有 0.05% Tween 20 之 PBS 洗二次，然後加入 50 μl 連有 biotin 之抗 IL-2 或 IL-4 cytokines 之 monoclonal antibodies。培養 37^{o}C，90 分後，再洗四次，然後加入 poly-horse radish peroxidase conjugated avidin。培養 37^{o}C，90 分後，洗四次，最後加入 substrate (4mg 3-amino-9-ethyl carbazole + 1ml dimethylformamide + 14ml acetic buffer, pH 5)。若細胞有 cytokines 分泌，則有呈色反應。用水沖洗以中止反應，乾燥後，以顯微鏡計算呈色點數 (此即 cytokines 分泌細胞數目)。結果發現，在同時有 silymarin 與 PHA 的刺激下，平均 IL-2 反應點數爲只有 PHA 組之 2.3 倍；而平均 IL-4 反應點數爲只有 PHA 組之 1.5 倍。Silymarin 在 LPS 刺激下可增強巨噬細胞對 sarcoma cells 破壞能力，且巨噬細胞以 silymarin 併以適當濃度之 LPS 處理後與淋巴細胞培養可促使後者增生。以 ELISA 可證實 IL-1 分泌有增加。

總結：Silymarin 本身對淋巴細胞有直接刺激效果，在同時有 PHA 存在下刺激效果會更好。同時，silymarin 有助於受刺激細胞 Th1 及 Th2 型之反應，但前者效果比後者好。Silymarin 在 LPS 刺激下可增強巨噬細胞活性。

四、計畫結果自評

本計畫證實 silymarin 活性類似維生素 E，亦爲免疫調控物質，可刺激淋巴球並提升其 Th1-cytokines 之釋放，同時也可刺激巨噬細胞。若果真如此，則除了可解釋 silymarin 對肝臟的功效，將來亦可進一步求證 silymarin 是否對其他細胞內病原感染亦有保護效果，因細胞內感染保護性免疫以 Th1 型的細胞免疫反應爲主。

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