行政院國家科學委員會專題研究計畫　成果報告

開發可應用於化妝品的抗皮膚老化之成分及研究其分子作用機轉(II)

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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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※　　**及研究其分子作用機轉(II)**　　※

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計畫類別：☑個別型計畫　　□整合型計畫

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共同主持人：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：嘉南藥理科技大學

中　華　民　國　93 年　9 月　23 日

Abstract:

中文摘要。

日光紫外線的暴露引起皮膚細胞的傷害，進而影響到皮膚的緊縮性及彈性，並且會誘發皮膚提前老化現象，這可能是因膠原蛋白含量的減少。紫外線所引起的皮膚改變，已知是一種氧化性傷害藉著活化及增加細胞內的 PKC 的活性，進而促進 Matrix metalloproteinases (MMPs)生合及造成膠原蛋白的分解。重複性的紫外線照射已知會引起持續性的增加 MMP 基因的表現。綠藻是一種淡水單胞藻,在日本及台灣食用已有多年的歷史，其相關生理功能包括有降血脂、降血糖、增加免疫力等。運用綠藻的萃取物當作緩和皮膚老化在上市的化妝品已有先例，然而卻無任何相關的研究報告可查證。我們將利用已知的促皮膚老化的方式，及不同年齡層的皮膚細胞株進行綠藻對皮膚抗老化的功能性及機轉的探討。**91 年的計畫(NSC 91-2626-B-041-001)**已證實綠藻萃取物可減少 MMP 誘導物對於 MMP 產量的影響。而 TIPM 的產量也會因細胞添家有綠藻萃取物而增加。計畫過程中我們也發現綠藻的萃取物對於 UVA 所引起的細胞死亡具有保護作用。UVA 引起細胞凋亡可因細胞膜死亡接受器的活化或自由基對於粒線體的破壞所致。因此，本年度計畫除延續探討綠藻萃取物對於紫外線對皮膚細胞膠原蛋白的分解的影響之外，也將探討綠藻萃取物預防 UVA 所引起的細胞死亡的可能路徑。

英文摘要

Solar UV radiation damages human skin, affecting skin tone and resiliency and leading to premature ageing (photoaging) (Gilchrest &Yaar, 1992). Oxidative stress caused by UV radiation, ozone, hydrogen peroxide & free radicals are known o increase PKC activity. Skin damage by oxidants may lead to activation of PKC and AP-1, thus increasing Matrix metalloproteinases (MMPs) expression and collagen degradation. UV radiation activates cell surface growth factor and cytokine receptors, and therefore mimics the actions of receptor ligands (Warmuth *et al*, 1994; Sachsenmaier *et al*, 1994; Rosette & Marin 1996; Huang *et al.*, 1996; Bender *et al*, 1997).

Chlorella, a type of unicellular fresh water growth algae, has been a popular foodstuff in Japan and Taiwan. There are many studies have shown some beneficial effects of *Chlorella* administration, such lowering blood lipid profiles (Sano &Tanaka, 1987；Okuda *et al.*, 1975 ； Sano *et al.*, 1988 ； Yang *et al.*, 2001) ； ameliorating hyperglycemia (Rodriguez-Lopez M. & Lopez-Quijada 1971; 李宏圖等人 1977)； increasing function of immune system (Singh *et al.*, 1998; Tanaka *et al.*, 1984; Tanaka *et al.*, 1998; Konishi *et al*., 1996).

Introduction:

運用綠藻的萃取物當作緩和皮膚老化在上市的化妝品已有先例，雖然產品本身標榜具有此一功效卻無任何相關的研究報告可查證。經我們初步的實驗結果證實綠藻粗萃取物與 MMP inducers 同時加入培養液中可減少 MMP-1 的產量（請見圖一）。 因此，我

們將利用已知的促皮膚老化的方式，即以 UVA2 照射人的皮膚的細胞(human skin fibroblasts)或 MMPs 的誘發藥物（如 IL-1β + PDGF-BB 或 PMA）去促進 MMPs 的生成的方式。再比較以純化後的綠藻萃取物處理後的皮膚的細胞的 MMPs 產量來當作初步的證實。同時，我們也將比較不同年齡來源的細胞株的 MMPs 產量，以及這些細胞株對於綠藻萃取物的反應。接著再逐一探討萃取物的成分及緩和皮膚老化的可能分子機轉，如利用 Western blotting 觀察 Type I & III procollagen 及 PKC-α蛋白質的量經過不同的 MMP inducers 及純化的綠藻萃取物處理後的變化；利用 immunopercipitation 沉澱 ERK 再以 Western blotting 測量 ERK；利用 Reverse trascriptase-polymerase chain reaction (RT-PCR): to analyze the expression of MMP-1, MMP-3, TIMP-1, TIMP-3 mRNA in skin fibroblast stimulated by MMP inducers, UV, or pre-treated with extracts of Chlorella；利用 XTT kits 來偵測皮膚細胞無血清培養液及加入純化的綠藻萃取物的細胞分化速率。

運用綠藻的萃取物當作緩和皮膚老化在上市的化妝品已有先例，雖然產品本身標榜具有此一功效卻無任何相關的研究報告可查證。因此，**91 年的計畫(NSC 91-2626-B-041-001)**中我們利用 UVA 及 UVB 照射人的皮膚的細胞(human skin fibroblasts)去促進 MMPs 的生成的方式來比較皮膚細胞經綠藻的萃取物處理後的 MMPs 產量來當作初步的證實。接著再逐一探討萃取物的成分及緩和皮膚老化的可能分子機轉。長期的紫外光曝曬引起大量的 mtDNA mutations，這與皮膚老化有相關性而主要的 mutations 是 common deletion（即在 4977 鹼基的位置缺乏配對）。這也是粒線體突變的一中生物指標 在許多老化的組織中都可發現有大量的 common deletion 的囤積(Birch-Machin et al, 1998; Berneburg et al, 1997; Yang et al, 1995)。根據實驗結果顯示，經綠藻萃取物處理後的皮膚細胞經過 UVA 及 UVB 照射後的存活率明顯的比控制組要高的多。因紫外線引起的細胞凋亡（apoptosis）原因之一是因為引起細胞膜上的死亡接受器（CP95）進而活化 caspase-8 然後在繼續活化 caspase-3 及 Bid protein（Li *et al*, 1998; Luo *et al* 1998）。後者啟動 Bcl-2 family 而引起 *cytochrome c* 自粒線體釋放出來造成細胞的死亡(Desagher et al, 1999）。另外，紫外線引起的細胞凋亡也可經由 reactive oxygen species（ROS）引起 *cytochrome c* 的釋放而造成細胞凋亡（Kulms *et al* 2002）。因此除了繼續研討綠藻萃取物影響 MMPs 產量的分子機轉外，其增加細胞存活的機轉亦是值得加以探討的主題。

Methods:

Water extract of Chlorella (WEC)是由綠藻乾粉經由樂水萃取再加以過濾及冷凍乾燥保存。In order to verify the different response of UV exposure in different age-derived cell-line, various skin fibroblast cell-lines were used in this study. 1059 SK, 1090SK, and 966SK cell-line represented 20, 46 and 78 years old female skin, repectively.

Cell lines were exposed to long wave UV radiation for various period of time as control groups. In treated groups, cells were added WEC (0.1, 0.2, or 0.3 mg/ml), Vitamin E (0.25μM) or Vitamin C (150μM).

利用 Reverse trascriptase-polymerase chain reaction (RT-PCR): to analyze the expression of MMP-1, MMP-3, TIMP-1 TIMP-3 mRNA in skin fibroblast stimulated by MMP inducers, UV, or pre-treated with extracts of *Chlorella*。TIMP1 與 TIPM3 可中和 MMP-1 與 MMP-3 對於 type I 及 III 膠原蛋白之作用。因此，綠藻萃取物對於 UV 照射

對 TIMP1 與 TIPM3 的影響亦在此研究範圍之中。

Results:

Figure 1

Young skin -derived cells (20years old females) did not have significant change in MMP production after 1 h long wave UV radiation exposure. Addition of WEC (0.1, 0.2, or 0.3mg/ml) decreased the production of MMP1, whereas addition of vitamin C or E did not affect MMP1 level.

Figure 2

Long wave UV exposure for 1 hr did not affect 1090SK cell line, however, the addition of 0.2 and 0.3 mg/ml of WEC decreased the MMP1 production significantly ($p<0.05$ and 0.01, respectively). Vitamin C and E had no effect on MMP1 production after UV exposure.

Figure 3

After prolong UV exposure to 2 hr, MMP1 production was higher in control and non-exposure cells (shown as blank, $p<0.05$). All WEC doses significantly prevented MMP1 induction by UV exposure ($p<0.005$). Neither vitamin C not E could prevent the increase production of MMP1.

Figure 4

After exposure to UV for 1.5h, 966SK produced a higher MMP1 activity in control group than in blank ($p<0.05$). All WEC doses significantly prevented MMP1 induction by UV exposure ($p<0.005$). Neither vitamin C not E could prevent the increase production of MMP1.

Figure 5

MMP1 mRNA levels were not affect by UV exposure for 1h, nor affected by addition of WEC.

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