

The effect of Chinese herbal medicines on TNF- α induced matrix metalloproteinase-1, -9 activities and interleukin-8 secretion

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ABSTRACT. Matrix metalloproteinases (MMPs) play an important role in normal physiological functions and pathological processes. They are involved in normal skin functions as well as in the aging, healing, embryonic development, reproduction, and inflammatory responses of skin. Previous studies report that both high MMP-1 and MMP-2 activities were found in the skin of patients with dermatitis, and large amounts of MMP-9 have been reported to be accumulated in unhealed wounds. Interleukin-8 (IL-8), a C-X-C chemokine, may mediate neutrophil recruitment and activation and is involved in various inflammatory skin diseases. In this study, eleven Chinese herbal medicines were analyzed for their potential as anti-inflammatory agents using human fibroblast WS-1 cell lines. The results indicate MMP-1 and -9, but not MMP-2, were induced by TNF- α treatment in WS-1 cells. However, when WS-1 cells were pre-treated with eleven Chinese herbal medicines before TNF- α stimulation, all these herbal medicines suppressed TNF- α -stimulated MMP-1 activity in WS-1 cells as analyzed by casein zymography. In addition, the suppression of MMP-9 activity was also observed when WS-1 cells were treated with either *Paeonia suffruticosa*, *Scutellaria baicalensis*, *Saposhnikovia divaricata*, *Dioscorea opposita*, *Rubus chingii*, or *Salvia miltiorrhiza*. Of which, *R. chingii* significantly inhibited IL-8 secretion induced by TNF- α treatment as well. These results revealed that some novel components present in these Chinese herbal medicines may be used for the treatment of inflammatory responses in skin cells.

Keywords: Chinese herbal medicines; MMP-1,-2,-9; IL-8.

INTRODUCTION

Herbal remedies used in traditional folk medicines provide an interesting and yet largely unexplored source for the discovery and development of potential new drugs. Moreover, traditional medicinal methods still play vital roles in serving basic health needs in developing countries. Therefore, it is of great interest to screen these plants in order to validate their use in folk medicine while at the same time seeking to reveal their active principles vis-a-vis the isolation and characterization of their constituents.

Chinese herbal medicines (CHMs) are considered useful for the treatment of a variety of human deficiencies. CHMs are classified into many categories, and these include heat-clearing, blood-regulating, *Qi*-regulating, drain-dampening, wind-damp-dispelling, and exterior releasing (Chongyun et al., 2005). The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that play a key role in the turnover of the extracellular matrix in skin (Fisher et al., 1996). Aging and exposure to environmental insults, such as ultra-violet (UV) irradiation, increase the expression of MMPs (Fisher et al., 1998; Brenneisen et al., 2002). Excessive MMP activity, which causes the collapse of the meshwork in the extracellular matrix, produces UV irradiation-like skin damage, such as wrinkling, a loss of elasticity, and the dilation of surface micro-capillary vessels (Bologna, 1993). MMP-1, which belongs to the subfamily of collagenases, is the key enzyme in the

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collagen turnover that is required for remodeling of the dermal matrix. MMP-2, and -9, also known as gelatinases A and B, are important in clearing collagen fragments generated by collagenases. MMP proteolysis is regulated by the actions of endogenous inhibitors such as the tissue inhibitor of metalloproteinase (TIMP), which is involved in the binding of the active sites of both active and latent MMPs, forming stable, but inactivated, enzyme-inhibitor complexes (Kahari and Saarialho-Kere, 1997).

Interleukin-8 (IL-8), which belongs to the C-X-C sub-family of chemokines, is a chemotactic attractant for both neutrophils and T cells. IL-8 and other chemokines can activate or regulate the inflammatory activity of skin diseases like atopic dermatitis (Kimata and Lindley, 1994; Hatano et al., 1999; Nomura et al., 2003; Park et al., 2006), psoriasis (Fukuoka et al., 1998b), and bullous pemphigoid (Inaoki and Takehara, 1998). IL-8 is produced not only by monocytes and lymphocytes, which are the major targets of the chemotactic activity of this chemokine, but also by epithelial cells, fibroblasts, keratinocytes, and endothelial cells (Schroder, 1995; Noso et al., 1996; Fukuoka et al., 1998a). IL-8 directly enhances MMP-2, -9 production on the endothelial cells which regulate angiogenesis (Li et al., 2003) and on the endometrial stromal cells which promote tumor invasiveness (Mulayim et al., 2004).

To date, the relationship between these Chinese herbal medicines and the MMP and IL-8 activities in human fibroblasts has been little investigated. In the present study, we chose the CHMs usually used to dispel pathogenic factors from the exterior of the body by diaphoresis, as well as those used in febrile or inflammatory conditions to invigorate blood circulation and for their tonic actions. Therefore, the aim of this study was to investigate the effects of CHMs on the regulation of MMP-1, -2, -9 as well as TIMP-1 activities and IL-8 secretion in TNF- α treated human fibroblast WS-1 cells.

MATERIALS AND METHODS

Materials

The tested Chinese herbal medicines (Table 1) were purchased from local medicinal markets in Taipei. Their identities were authenticated by Professor Chang, H.C. at the Bureau of Food and Drug Analysis, of the Department of Health in Taiwan. After authentication, the specimens were then deposited in the Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan.

Preparation of plant extracts

The dried leaves of the plants selected for this study were pulverized and extracted using 95% ethanol twice. After filtering, the combined filtrates were concentrated under reduced pressure. The final residues were then freeze-dried and stored in a closed container until use. We calculated the yields of plant extracts using the following formula:

Yield (%) = (mass of the extract/mass of the dried raw plant material) \times 100%.

Cell Culture

Human skin fibroblast WS-1 cells were obtained from CCRC 6003, Hsinchu, Taiwan. The cells were maintained in a 37°C, 5% CO₂ humidified incubator as monolayers in 75 cm³ culture flasks. They were grown in Minimal Essential Medium (MEM, Eagle) with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 10% inactivated fetal calf serum, 50 U/mL penicillin and 50 μ g/mL streptomycin. We cultured the cells until confluence and harvested them with a trypsin-EDTA solution. Subsequently, the WS-1 cells (1×10^5 cells/well in 0.5 mL medium) were distributed and cultured in 24-well plates. Samples of CHM (100 μ g/mL) were added to the cultures and incubated at 37°C for 48 h. After a 48-h incubation period, the medium was removed, and a fresh serum-free medium with 10 ng/ml of TNF- α in combination with CHMs (100 μ g/mL) was added for an additional 24-h period. Ultimately, the culture supernatants were collected and frozen at -70°C for further analysis while the cells were harvested for MTT assay.

MTT assay for cell viability

Mitochondrial dehydrogenase activity, which reduces 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, St. Louis, USA) in active mitochondria to purple formazan, was used to determine cell survival in a colorimetric assay. Cell viability was calculated accordingly:

Cell viability = Absorbance (sample tested) / Absorbance (medium only) \times 100

MMP activity by Zymography

Analyses of MMP-1 activity by casein zymography, MMP-2 and -9 activities by gelatin zymography were as described (Windsor, 2002) with minor modification. WS-1 cells were cultured and treated with eleven CHM (100 μ g/ml) separately for 48 h. After incubation, the culture media were replaced with a serum-free MEM containing 10 ng/ml of TNF- α and each of the CHMs for an additional 24 h. Five μ g of total proteins in the cultured serum-free media were subjected to SDS-PAGE analyses on 10% gels containing casein (for MMP-1 activity) or gelatin (for MMP-2 and -9 activity) substrate (1 mg/mL) under non-reducing conditions. Recombinant MMP-9 (0.5 ng) (Chemicon, Temecula, USA) were used as the standard control. After electrophoresis, the gels were washed with distilled water containing 2.5% Triton X-100 with gentle shaking for 1 h. The gels were then incubated at 37°C for 18 h in a buffer (50 mM Tris-HCl [pH 7.5], 0.2 M NaCl, 5 mM CaCl₂) and subsequently stained with Coomassie Blue. Clear bands of protein degradation were visualized by destaining in 30% methanol containing 10% glacial acetic acid. We then scanned photographs of the gel with an imaging densitometer system (Kodak 1D Image Analysis System 3.5). The MMP activity was calculated from

Table 1. Ethnobotanical data of the selected Chinese herbal medicines.

No.	Species	Family	Traditional uses (Xie and Huang, 1994)	Classification
1	<i>Paeonia suffruticosa</i> Andr.	Ranunculaceae	It is used to eliminate heat from the blood for treatment of bleeding in high febrile conditions	Heat-clearing and blood-cooling agent
2	<i>Scutellaria baicalensis</i> Gergi	Labiatae	It is used to eliminate heat in the lung for cough with yellow thick phlegm, used for the treatment of pyogenic infections of skin, hypertension, and threatened abortion.	Heat-clearing and damp-drying agent
3	<i>Saposhinkovia divaricata</i> (Turcz.) Schischk	Umbelliferae	It is used as diaphoretic for affection due to wind and cold and rheumatic pain.	Wind-cold-effusing agent
4	<i>Dioscorea opposita</i> Thunb	Dioscoreaceae	It is used to invigorate the functions of spleen and stomach for the treatment of poor appetite and chronic diarrhea.	Qi-supplementing agent
5	<i>Rubus chingii</i> Hu	Rosaceae	It is used as an astringent for the treatment of frequent micturition and seminal emission.	Astringent agent
6	<i>Angelica sinensis</i> Diels	Umbelliferae	It is used to nourish the blood and to invigorate the blood circulation for the treatment of menstrual disorders.	Blood-nourishing agent
7	<i>Angelica dahurica</i> Benth	Umbelliferae	It is used in the treatment of affection due to wind, cold, or dampness, as anodyne for frontal headache, etc.	Wind-cold-effusing agent
8	<i>Glycyrrhiza uralensis</i> Fisch.	Leguminosae	It is used to invigorate the functions of the heart and spleen for the treatment of symptoms due to deficiency of vital energy of these viscera; as antitoxicant for drug poisoning.	Qi-supplementing agent
9	<i>Ligusticum chuanxiong</i> Hort.	Umbelliferae	It is used to invigorate blood circulation and promote the flow of vital energy for the treatment of abnormal menstruation, dysmenorrheal, amenorrhea, and coronary heart diseases.	Blood-quickening and stasis-dispelling agent
10	<i>Astragalus membranaceus</i> (Fisch.)	Leguminosae	It is used to replenish the vital energy and to stop perspiration for the treatment of spontaneous perspiration, night sweat, prolapse of uterus and anus.	Qi-supplementing agent
11	<i>Salvia miltiorrhiza</i> Bge	Labiatae	It is used to promote blood circulation and to remove blood stasis for the treatment of dysmenorrheal, amenorrhea, abdominal masses due to stagnation of blood, carbuncles, and ulcers.	Blood-quickening and stasis-dispelling agent

the intensity value (area \times intensity) for each band by Kodak 1D Image Analysis Software and normalized with the intensity value with the medium alone and multiplied by 100%.

For an inhibition percentage calculation, the band intensity for the CHM treated group was normalized with the TNF- α treated group and shown as relative to the control (TNF- α) activity.

IL-8 and TIMP-1 analysis by enzyme-linked immunosorbent assay (ELISA)

The WS-1 cells were treated as described above. Concentrations of IL-8 and TIMP-1 in the supernatants were quantified using a commercially available duoset ELISA development system (R&D Systems; Minneapolis, MN). In principle, ELISA plates were coated with specific

mouse anti-human IL-8 antibody (4 μ g/ml) or mouse anti-human TIMP-1 antibody (2 μ g/ml). Properly diluted cell-free supernatants were then added to the wells in duplicate and incubated for 2 h, after which time, the secondary biotinylated goat anti-human IL-8 antibody (20 ng/ml) or goat anti-human TIMP-1 antibody (50 ng/ml) were added. After washing to remove unbound reagent, streptavidin-conjugated horseradish-peroxidase was added and incubated for 20 min. After washing, a 1:1 solution of H₂O₂ and tetramethylbenzidine was added and developed until the desired color was reached. The intensity of the color was measured at 450 nm in an ELISA plate reader (Emax, Molecular Device). The data was then calculated according to a standard curve using a Softmax computer program. The detecting sensitivity for IL-8 and TIMP-1 ranged from 31.25 to 2000 pg/ml.

Data Analysis

The data of each triplet test was presented as the mean \pm standard error bar (SEM). The student's *t*-test was used to analyze the significance of differences between the non-TNF- α treated group control (medium alone) and the TNF- α -treated group and between the CHM-treated groups and the TNF- α -treated group (relative to control). A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Chinese herbal medicines do not affect WS-1 cell viability

The MTT assay was performed to investigate whether Chinese herbal medicines affect the growth of WS-1 cells. The viabilities of human WS-1 cells at 100 $\mu\text{g/ml}$ of these CHM extracts were assayed by MTT. As shown in Figure 1, the cell viabilities after being treated with each of the eleven CHM were above 80% at the examined concentration, indicating no significant toxic effects as compared to the control (100%).

Chinese herbal medicines inhibit MMP-1 activity

To further investigate the potential anti-aging effects of Chinese herbal medicines on WS-1 cells, the MMP-1 activity was analyzed by casein zymography. As shown in Figure 2A, TNF- α significantly up-regulated MMP-1 activity ($p < 0.001$) as compared to the medium alone. All the tested CHMs at 100 $\mu\text{g/ml}$ concentration suppressed

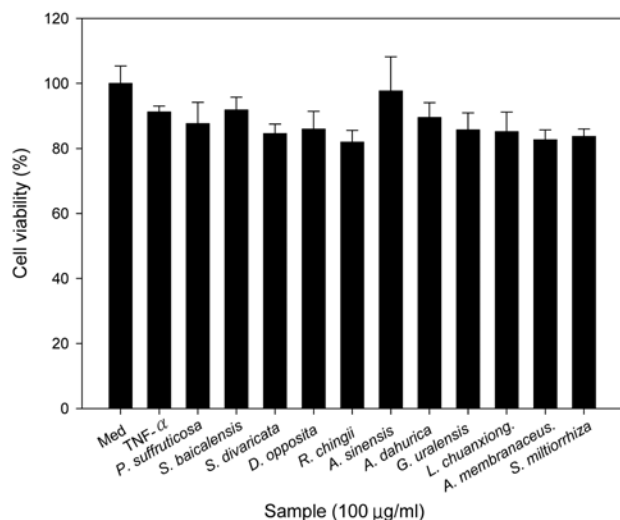


Figure 1. Effect of Chinese herbal medicines on WS-1 cell viability. WS-1 cells were cultured and treated with eleven CHMs (100 $\mu\text{g/ml}$) separately for 48 h. After incubation, the culture media were replaced with a serum-free MEM containing 10 ng/ml of TNF- α and each of the CHMs for an additional 24 h. Cell viability was determined according to the MTT assay as described in Materials and Methods. Results are expressed as mean \pm standard error of three independent experiments.

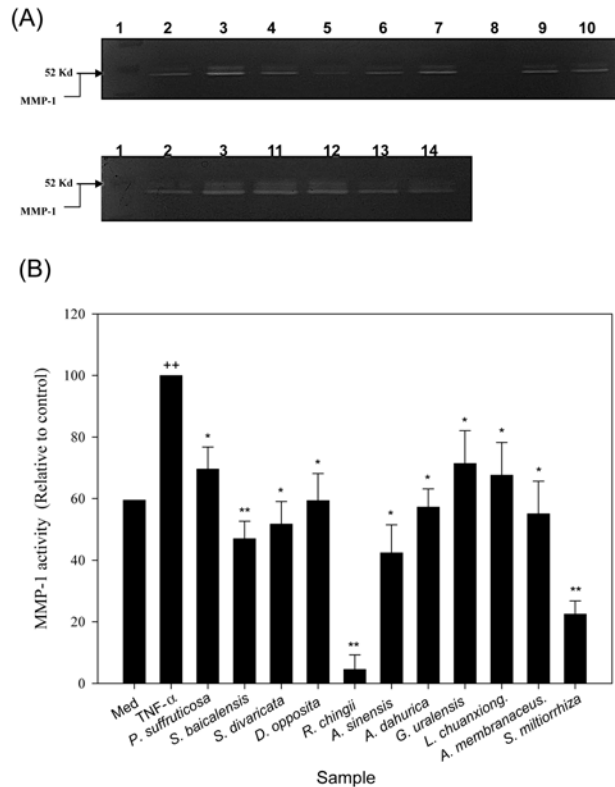


Figure 2. Effect of Chinese herbal medicines on MMP-1 activity by casein zymography gel analysis. WS-1 cells were cultured and treated with eleven CHMs (100 $\mu\text{g/ml}$) separately for 48 h. After incubation, the culture media were replaced with a serum-free MEM containing 10 ng/ml of TNF- α and each of the CHMs for an additional 24 h. The supernatants were used for MMP-1 activity assay by casein zymography gel as described in Materials and Methods. (A) Lanes 1-14 represent the results of molecular weight marker, medium alone without treatment, TNF- α treatment, and eleven CHM treatments, respectively; (B) The MMP-1 activity was semi-quantified by densitometer and represented as its value of treated groups over that of TNF- α treated cells (relative to control). ++ represents $p < 0.001$ as compared to medium alone (non-TNF- α treated cells); * and ** represent $p < 0.05$ and < 0.001 , respectively, as compared to TNF- α -treated cells. Values are expressed as mean \pm standard error of six independent experiments.

the TNF- α -induced MMP-1 activity of WS1 cells to a varying degree. The inhibitory effect on the MMP-1 activity was semi-quantified and expressed as shown in Figure 2B. Among which, *R. chingii* exhibited the most significant inhibitory activity ($94.46 \pm 4.73\%$, $p < 0.001$), followed by *S. miltiorrhiza* ($77.50 \pm 4.28\%$, $p < 0.001$), *A. sinensis* ($57.58 \pm 9.05\%$, $p < 0.05$), *S. baicalensis* ($52.97 \pm 5.61\%$, $p < 0.001$), *S. divaricata* ($48.26 \pm 7.31\%$, $p < 0.05$), *A. membranaceus* ($44.92 \pm 10.57\%$, $p < 0.05$), *A. dahurica* ($42.76 \pm 5.92\%$, $p < 0.05$), *D. opposita* ($40.62 \pm 8.78\%$, $p < 0.05$), *L. chuanxiong* ($32.37 \pm 10.62\%$, $p < 0.05$) and *P. suffruticosa* ($30.39 \pm 7.14\%$, $p < 0.05$). In contrast, *G. uralensis* had a minimal effect ($29.22 \pm 10.23\%$, $p < 0.05$).

Effect of Chinese herbal medicines on MMP-2 and 9 activities

The MMP-9 and -2 activities were measured by gelatin zymography. As shown in lane three of Figure 3A, the TNF- α treatment significantly increased MMP-9 ($p < 0.05$), but not MMP-2, activities as compared to the medium alone. Among the eleven Chinese herbal medicines tested, six extracts showed different degrees of an inhibitory effect on the MMP-9 activity of TNF- α -stimulated cells, including *R. chingii* ($59.23 \pm 10.94\%$, $p < 0.05$), *S. baicalensis* ($56.83 \pm 3.71\%$, $p < 0.05$), *S. divaricata* ($51.96 \pm 10.67\%$, $p < 0.05$), *S. miltiorrhiza* ($42.33 \pm 10.35\%$, $p < 0.05$), *P. suffruticosa* ($28.93 \pm 10.06\%$, $p < 0.05$), and *D. opposita* ($24.23 \pm 7.69\%$, $p < 0.05$). These results are shown in Figure 3B. However, only *S. divaricata* ($52.11 \pm 12.98\%$, $p < 0.05$) and *R. chingii* ($50.30 \pm 7.16\%$, $p < 0.05$) showed a significant inhibitory effect on the MMP-2 activity of cells treated with TNF- α alone (Figure 3).

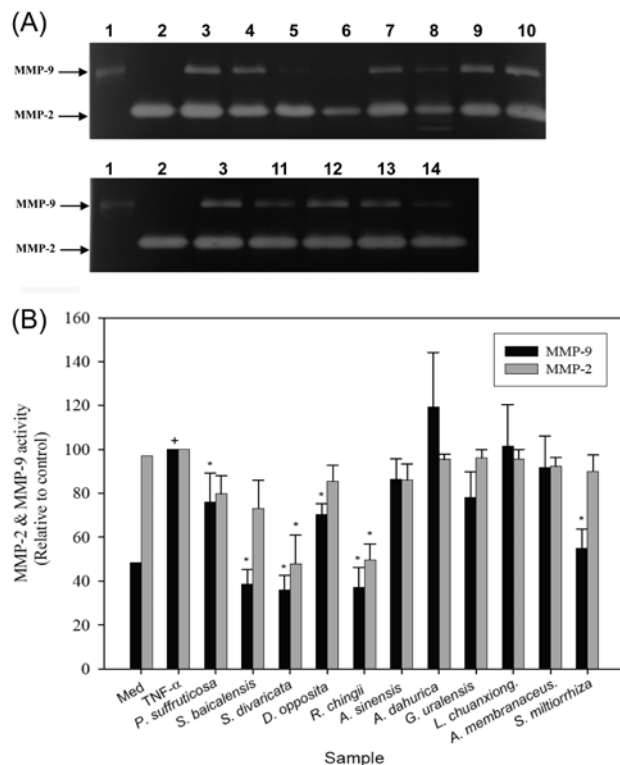


Figure 3. Effect of Chinese herbal medicines on MMP-2 and -9 activities analyzed by gelatin zymography gel. WS-1 cells were cultured and treated as described in Figure 2. The supernatants were used to determine the MMP-2 and -9 activities by gelatin zymography gel analysis. (A) Lanes 1-14 represent recombinant MMP-9 (0.5 ng) treatment, medium alone without treatment, TNF- α treatment, and cells treated with each of the eleven CHMs, respectively; (B) The MMP-2 and -9 activities were semi-quantified by densitometer and represented as the values of treated groups over that of TNF- α treated cells (relative to control). + represents $p < 0.05$ as compared to medium alone (non-TNF- α treated cells); * represents $p < 0.05$ as compared with the levels of TNF- α -treated cells. Values are expressed as mean \pm standard error of six independent experiments.

Effect of Chinese herbal medicines on TNF- α -induced TIMP-1 secretion

TNF- α did not have a significant effect on the TIMP-1 production of WS-1 cells as shown in Figure 4. Among the eleven samples tested, both *A. dahurica* and *L. chuanxiong* significantly increased TIMP-1 secretion by 1.29 fold and 1.78 fold, respectively. While *R. chingii* showed significant inhibition, *P. suffruticosa*-, *S. baicalensis*-, and *S. divaricata*-treated cells showed an inhibitory trend in the TIMP secretion.

Effect of Chinese herbal medicines on TNF- α -induced IL-8 secretion

IL-8 secretion was significantly enhanced in the TNF- α -stimulated WS-1 cells as compared to the control cells. When treated with the eleven Chinese herbal medicines, the TNF- α -induced IL-8 secretion was downregulated dramatically by $64.30 \pm 7.25\%$ ($p < 0.05$) in *R. chingii*-treated cells. In contrast, a synergistic/additive stimulated effect was detected in the groups treated with *P. suffruticosa* and *S. divaricata*.

DISCUSSION

Recent evidence has indicated that chronological aging and UV irradiation alter the signal transduction pathways of human skin, promote MMPs expression, decrease pro-collagen synthesis, and cause connective tissue damage (Herrmann et al., 1993; Brenneisen et al., 1996; Chung et al., 2001; Fisher et al., 2001; Kang et al., 2001; Brenneisen et al., 2002; Chung et al., 2002; Rittie and Fisher, 2002; Choi et al., 2007). These results indicate that the downregulation of MMPs activities could be beneficial to human skin and have great commercial potential. MMP-1 is the most abundant MMP produced in fibroblast cells. Studies conducted by many laboratories indicated that collagen synthesis becomes lower with increased age, but MMP-1 levels become higher in sun-protected human skin *in vivo* (Varani et al., 2000; Moon et al., 2006). In this study, we examined eleven CHMs and found that all the tested samples have inhibitory effects on TNF- α -induced MMP-1 activity of WS-1 cells. Notably, *R. chingii* exhibited a potent inhibition of TNF- α -induced MMP-1, -2, and -9 activities. In addition, the secretion of TIMP-1 and IL-8 induced by TNF- α treatment was also inhibited by *R. chingii* extract using ELISA analysis. The inhibitory effect on TNF- α induced MMP-9 activity was also observed in the treatment of *R. chingii*, *S. baicalensis*, *S. divaricata*, *S. miltiorrhiza*, *P. suffruticosa*, and *D. opposita* as measured by gelatin zymography. However, we were unable to detect any difference among the total MMP-9 protein expression in cells treated with various tested Chinese herbal medicines by ELISA analysis as observed by gelatin zymography method (data not shown). Nonetheless, our results indicate that the inhibition of MMP activities by these tested CHMs might be a potential strategy for the prevention and/or the treatment of UV-induced skin damage.

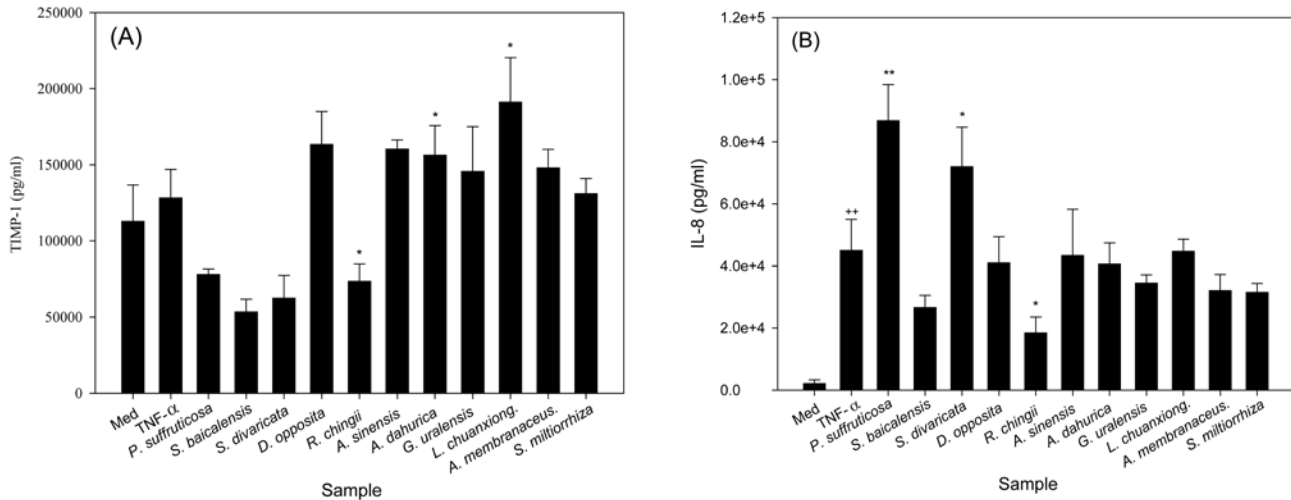


Figure 4. Effect of Chinese herbal medicines on TIMP-1 and IL-8 secretion by ELISA. WS-1 cells were cultured and treated as described above. The supernatants were used to assay the TIMP-1 (A) and IL-8 (B) secretion by ELISA as described in Materials and Methods. The results are expressed as mean \pm standard error of 3-5 independent experiments. ++ represents $p < 0.001$ as compared to medium alone (non-TNF- α treated cells). * and ** represent $p < 0.05$ and < 0.001 , respectively, as compared to TNF- α -treated cells.

Several studies have indicated that UV-irradiation increases MMPs activities while the TIMP-1 synthesis is not altered in cultured human fibroblast (Herrmann et al., 1993; Brenneisen et al., 2000; Naru et al., 2005). Hence, unbalanced synthesis of MMP and TIMPs may promote proteolysis and lead to cutaneous photoaging. Our study shows that TNF- α does not regulate TIMP-1 secretion of WS-1 cells. *A. dahurica* and *L. chuanxiong* significantly enhanced TIMP-1 expression which may contribute to further inhibition of MMP-1 activities. This stimulatory effect was not seen in WS-1 cells treated with the other herbal medicines. *Rubus chingii* even showed a significant inhibitory effect on TIMP-1 expression. Thus, *A. dahurica* and *L. chuanxiong* might contribute to anti-aging effects on the human skin fibroblast, and they might have potential as new ingredients in natural cosmetics.

We also evaluated the effects of CHMs on IL-8 production by TNF- α -stimulated WS-1 cells. The IL-8 level was significantly increased in the psoriatic patients compared with the control (Okubo and Koga, 1998). The IL-8 was expressed in the neutrophils of psoriasis (Duan et al., 2001). TNF- α is well known to stimulate IL-8 production by human dermal fibroblasts (NHDF) as reported by Fukuoka et al. (Fukuoka et al., 1998a). Studies have also demonstrated that IL-8 expression enhances angiogenic activity through the induction of MMP-9 and subsequently regulates the tumorigenesis and production of spontaneous metastases of human transitional cell carcinoma and human prostate cancer (Inoue et al., 2000a; Inoue et al., 2000b). Moreover, an anti-IL-8 antibody inhibits the growth of bladder cancer xenografts via the downregulation of MMP-2 and MMP-9 expression (Mian et al., 2003). Among the Chinese herbal medicines tested in this study, *R. chingii* significantly downregulated IL-8 secretion (Figure 4B). It is possible that *R. chingii* also inhibits MMP-2

and MMP-9 expression in WS-1 cells via the similar mechanisms triggered by the anti-IL-8 antibody. Taken together, these data suggest that *R. chingii* may inhibit the migration of neutrophils into skin lesions by reducing the IL-8 autocrine and/or paracrine functions which are secreted by dermal fibroblasts and keratinocytes. It is noted that *P. suffruticosa* and *S. divaricata* promoted IL-8 secretion significantly, but inhibited MMP-1 and 9 activities. These results suggest that the IL-8 may modulate the MMPs' expression indirectly. Furthermore, IL-8 secretion and MMP activities may not be positively co-related due to the major compounds interacting synergistically or antagonistically.

Rubus chingii has traditionally been used for the treatment of frequent micturition, uorrhoea, and seminal emission (Xie and Huang, 1994). In a recent literature, *R. chingii* was reported to protect against tert-Bu hydroperoxide-induced oxidative damage in primary rat hepatocytes (Yau et al., 2002). It is also reported to decrease the contents of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prostaglandin E₂ as well as increase the amount of LHRH secreted by the thymus gland and the level of testosterone in blood (Chen et al., 1996). *Rubus chingii* extract also showed an inhibitory effect on tyrosinase activity in rats (Hwang and Lee, 2007). Traditionally, *S. divaricata* has been used as a diaphoretic for effects related to the wind and cold and for rheumatic pain as well as a spasmolytic for tetanus (Xie and Huang, 1994). The literature shows that *S. divaricata* exhibits anti-proliferative properties against several human tumor cell lines as well as potent antioxidant, anti-inflammatory, and protective properties on LPS-activated RAW 264.7 cells (Chang et al., 2007; Tai and Cheung, 2007; Chang et al., 2007). It also exhibited the effects of anti-fever and analgesia, sedation, antibacterial effects, regulation of immunological function, anticoagulation, and others (Gao, 2004). *A.*

dahurica inhibited cyclooxygenase-2 (COX-2) expression (Ban and Ohuchi, 2003) and had the potential to modulate cytochrome P450 activity (Ioannides, 2002; Saita, 2006). *Ligusticum chuanxiong* was thought to activate the blood, promote qi, expel wind and relieve pains (Zhang, 2005). It was also used to treat cardiovascular and cerebrovascular diseases (Fang, 2006; Lim and Yong, 2004; Wang and Ou-Yang, 2005; Wang et al., 2006). However, despite the many medical applications that have been described, nothing in the literature has previously investigated the effects of *R. chingii*, *S. divaricata*, *A. dahurica*, and *L. chuanxiong* on skin fibroblast cells. To the best of our knowledge, the present study is the first to show the differential regulatory effects of the extracts from *R. chingii*, *S. divaricata*, *A. dahurica* and *L. chuanxiong* on human fibroblast WS-1 cells. These results may justify the search for active components from these specimens, which may be used for treating the inflammatory responses of skin cells.

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中草藥對腫瘤壞死因子- α 所誘發之基質金屬活化蛋白酶-1 及 -9 之活性及介白素-8 分泌之影響

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基質金屬活化蛋白酶 (matrix metalloproteinases, MMPs) 在正常生理功能及病理狀態下扮演非常重要之角色。它們與正常皮膚功能以及老化、傷口癒合、胚胎發育、生殖和皮膚之發炎反應都有關係。先前的研究報告指出，在皮膚炎病人之皮膚上，發現高量的基質金屬活化蛋白酶-1 和-2 之活性；在未癒合的傷口上，聚積著大量的基質金屬活化蛋白酶-9。介白素-8 (Interleukin-8, IL-8)，屬於 C-X-C 趨化素，它可以調節嗜中性球之召集與活化，而且參與各種發炎反應之皮膚疾病中。在此研究中，我們利用人類纖維母細胞株 WS-1，分析 11 種具潛力之抗發炎反應之中草藥。結果顯示，當我們以腫瘤壞死因子- α (TNF- α) 刺激 WS-1 細胞時，基質金屬活化蛋白酶-1 和-9 (而非基質金屬活化蛋白酶-2) 會被誘導增加。但當我們在 WS-1 細胞中這 11 種中草藥做前處理，再以腫瘤壞死因子- α 刺激，所有的中草藥皆可抑制 WS-1 細胞中由腫瘤壞死因子- α 所誘導之基質金屬活化蛋白酶-1 之活性。此外，若先以牡丹皮 (*Paeonia suffruticosa*)，黃芩 (*Scutellaria baicalensis*)，防風 (*Saposhnikovia divaricata*)，山藥 (*Dioscorea opposita*)，覆盆子 (*Rubus chingii*)，或丹參 (*Salvia miltiorrhiza*) 前處理 WS-1 細胞，則皆可抑制基質金屬活化蛋白酶-9 之活性。當中，以覆盆子 (*Rubus chingii*) 前處理 WS-1 細胞，可以顯著的抑制腫瘤壞死因子- α 所誘導之介白素-8 之分泌。這些結果顯示，在這此 11 種中草藥中，可能存在著一些有效成分，可以用來治療皮膚細胞之發炎反應。

關鍵詞：中草藥；基質金屬活化蛋白酶-1, -2, -9；介白質素-8。

