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Synthesized pyridine compound derivatives decreased TNF alpha and adhesion molecules and ameliorated HSV-induced inflammation in a mouse model

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ABSTRACT

Synthesized pyridine compound derivatives (SK94, SK126) from a natural lead source were administered to mice to test for possible anti-TNF alpha and anti-inflammatory activities. Lipopolysaccharide (LPS)-induced TNF alpha production was analyzed in the endothelial cells, Raw 264.7 cells, and serum of normal mice after treatment with SK compounds. These compounds were also orally administered to a herpes simplex virus (HSV)-induced Behcet's disease mouse model to investigate their anti-inflammatory therapeutic effect. TNF alpha production was inhibited in a dose-dependent manner in the SK94 treated cells. E-selectin, VCAM-1, and ICAM-1 mRNA levels were also down-regulated. Treatment with 30 mg/kg SK94 inhibited 55% of the TNF alpha production in LPS challenged Balb/c mice ($n=8$). SK94 and SK126 were administered to the Behcet's disease-like mice for five consecutive days and SK94 improved in five out of six mice (83%), while it only improved in one out of nine mice (11%) in the pH 1.2 saline (artificial gastric juice) group ($P<0.005$), four out of ten mice (40%) in the thalidomide group ($P<0.05$), and six out of seven (86%) in the SK126 group ($P<0.005$). Soluble ICAM-1 was inhibited by 23.8% in the sera of SK94 treated mice and by 34.6% in SK126 treated mice when compared to artificial gastric juice. Based on these findings, SK compounds could be candidates for clinical trials.

1. Introduction

Tumor necrosis factor-α (TNFα) is a potent paracrine and endocrine mediator of inflammatory and immune functions. TNFα over expression has been implicated in acute and chronic inflammatory diseases, such as septic shock, bowel disease, Crohn's disease, rheumatoid arthritis, atopic dermatitis, psoriasis, and Behcet's disease (Edwards, 2004). TNFα is primarily produced in T cells, polymorphonuclear cells, dendritic cells, and macrophages (Wallach and Kovalenko, 2009). In macrophages, TNFα gene expression is induced by physical, chemical, and biological stimuli that include ischemia, trauma, irradiation, viruses, bacteria, tumor cells, complement, and cytokines such as intreleukin-1β (IL-1β), intreleukin-2 (IL-2), interferon-γ (IFNγ), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage CSF (M-CSF), and TNFα itself (Brouckaert et al., 1993). TNFα is also known to induce other inflammatory cytokines including IL-6 (Brouckaert et al., 1993) and IL-8 (Williams et al., 2008). TNFα plays a central role in a variety of inflammatory responses (Van der Meide and Schellekens, 1996); therefore, developers of treatments for inflammatory disease have focused on inhibitors of TNFα production.

Behcet's disease is a chronic, multi-systemic disorder with arthritic, gastrointestinal, mucocutaneous, ocular, vascular, and central nervous system involvement. This disease has a chronic course with periodic exacerbations and progressive deterioration (Shimizu, 1979). Although the etiology of Behcet's disease is unclear, viral infection has long been postulated as one of the main factors. Since Hulusi Behcet first proposed a viral etiology (Behcet, 1937), the viral hypothesis has been verified by the detection of viruses in the saliva (Lee et al., 1996b), intestinal ulcers (Lee et al., 1993), genital ulcers (Bang et al., 1997; Lee et al., 1996a), and serum (Hamzaoui et al., 1990; Sánchez Román et al., 1992) of patients with Behcet's disease. Subsequent to this finding, herpes simplex virus (HSV) inoculation of the earlobes of ICR mice was found to induce the development of Behcet's disease-like symptoms (Sohn et al., 1998). Manifestations in mice following HSV inoculation included multiple symptoms such as oral ulcers, genital ulcers, skin ulcers, eye symptoms, gastrointestinal ulcers, arthritis, and neural involvement, as well as skin crusting. The frequency of these symptoms was similar to that of patients with Behcet's disease (Kim et al., 1988). Anti-viral drugs such as Famcyclovir have been used to treat Behcet's disease, but with limited success. Specifically, these drugs have had only 40% efficacy in a Behcet's

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disease mouse model, compared to a 60% success rate when used to treat HSV (Sohn et al., 2001). Anti-TNFα antibody, Infliximab, and soluble TNF receptor (Etanercept) have also been used to treat Behcet's disease patients.

The pyridine compound derivatives of the SK chemicals SK126 and SK94 (Fig. 1) are synthetic compounds based on gentianine, which is one of the major components of *Gentiana Macrophylla Radix*. SK126 was shown to effectively inhibit the production of inflammatory cytokines including TNFα in antigen presenting cells (Kang et al., 2008). In this study, low-molecular weight, orally administered TNF alpha inhibitors were tested for their ability to ameliorate HSV-induced inflammation in a Behcet's disease mouse model.

2. Materials and methods

2.1. In vitro analysis of SK-compounds for cell adhesion molecules

Human Umbilical Vein Endothelial Cells (HUVEC) were treated with SK compounds for 30 min and then stimulated with TNFα for 4 h. The cells were then harvested, after which the RNA was extracted and subjected to RT-PCR.

2.2. Animals, introduction of Behcet's disease symptoms and treatment of Behcet's disease mice with placebo or SK-compounds

Four to five-week-old male ICR mice were used for this study. The earlobes of the mice were scratched with a needle and then inoculated with 1.0×10^6 plaque forming units/ml of HSV type 1 (F strain). Virus inoculation was conducted twice with a 10-day interval between treatments, after which the mice were observed for 16 weeks. Mice were bred in temperature- and light-controlled conventional rooms (20–22 °C, 12 h light cycle starting at 8:00 A.M.) with free access to food and water. During the experimental period, the animals were closely observed and photographed. Animals were handled in accordance to a protocol approved by our institutional animal care committee.

A revised Japanese classification with minor modifications was used to classify symptomatic mice with Behcet's disease. Briefly, oral, genital and other skin ulcers (including bulla and crust) and eye symptoms were classified as major symptoms, while arthritis, gastrointestinal ulcers and neurological disorders were identified as minor symptoms. Mice with at least one major and one minor symptom were classified as having Behcet's disease. Of the total number of HSV-injected mice, 15% developed Behcet's disease-like symptoms. Treatments that led to the disappearance of symptoms or a decrease in the lesion size of greater than 20% were classified as effective. Scoring of the severity of Behcet's disease was followed by determination of the Behcet's disease activity index, as outlined in the Behcet's disease Activity Form (www.behcet.ws/pdf/BehcetsDiseaseActivityForm.pdf). Among the symptoms in patients, mouth ulceration, genital ulceration, erythema, skin pustules, skin ulceration, joints-arthritis, diarrhea, red eye (right, left), reduced vision (right, left), loss of balance, discoloration, and swelling of the face were selected and analyzed in the Behcet's disease mouse model. The score of each symptom was one, and after the score was computed the total was used to determine the severity score of Behcet's disease. Symptomatic mice were photographed on the starting day of drug administration and once every week for five weeks on the same day of the week using a digital camera. SK compounds (1 mg) or thalidomide (50 μg) were orally administered to five to ten Behcet's disease-like mice for five consecutive days. Placebo was administered to ten Behcet's disease mice in an identical manner.

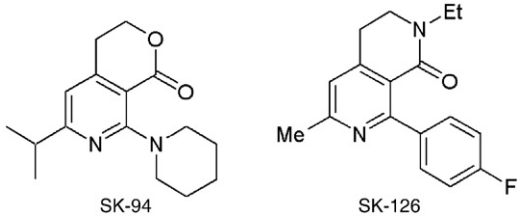

Fig. 1. Synthesized pyridine compound derivative structures (SK126, SK94). These are synthetic compounds based on gentianine, one of the major components of *Gentiana Macrophylla Radix*.

2.3. Preparation of SK126 and SK94

SK126 and SK94, which are pyridine derivatives based on lead compounds derived from a natural product, were provided by SK Chemicals (Suwon, Republic of Korea). Briefly, SK126, 2-ethyl-8-(4-fluorophenyl)-6-methyl-3,4-dihydro-2H[2,7] naphthyridin-1-one was synthesized using a five-step procedure. 2-Chloro-4,6-dimethylnicotinonitrile readily underwent Suzuki-coupling with 4-fluorophenylboronic acid to give 2-(4-fluorophenyl)-4,6-dimethyl-nicotinonitrile. Treatment of this coupled product with N,N-dimethylformamide dimethyl acetal followed by sulfuric acid yielded the cyclized compound, 8-(4-fluorophenyl)-6-methyl-2H-[2,7]naphthyridin-1-one. Finally, ethylation and hydrogenation of the lactam gave SK126, which was purified by silica gel column chromatography and subsequent recrystallization. NMR data describing the final compound revealed that the purity was greater than 95%.

2.4. ELISA

For detection of soluble ICAM-1, soluble VCAM-1, soluble E-selectin, soluble TNF receptor 1, and MCP-1, the drug treated mice were sacrificed on the last day of the drug treatment and the serum and spleens were collected for analysis. ELISA was applied using commercial kits for the detection of mouse soluble type 1 TNFα receptor (R&D system, Minneapolis, MN), TNFα (R&D system), soluble ICAM-1, soluble VCAM-1, soluble E-selectin (R&D system), and MCP-1 (R&D system). ELISA analysis was conducted on spleen tissue from each mouse. Briefly, the spleen was excised, finely minced with scissors, and then suspended in 3 mL of 10 mM potassium phosphate buffer (pH 6.0). The tissue samples were then homogenized and centrifuged at $9000\times g$. A micro BCA protein assay kit (Pierce, Rockford, Illinois, USA) was used to quantify each protein in the supernatants, after which ELISA was conducted according to the manufacturer's instructions. The ELISA reader was a Bio-Rad model 170–6850 microplate reader and the samples were read at a wavelength of 450 nm.

2.5. Administration of SK compounds to BALB/c mice

SK94 (1–70 mg/kg) or SK126 (0.1–100 mg/kg) were orally administered to BALB/c mice. Two hours after administration, mice were challenged intraperitoneally with LPS (1 mg/kg). Two hours after LPS challenge, blood was collected from the mice, and the sera were subjected to ELISA ($n=8$ for LPS-treated groups, $n=6$ for saline-treated group).

2.6. Statistical analysis

All data shown are the means ± S.D. Statistical differences between the experimental groups were determined according to a Student's *t* test and the Bonferroni correction. Statistical analyses were performed using MedCalc® version 9.3.0.0.

3. Results

3.1. mRNA expression of adhesion molecules were inhibited by SK compounds

Cell adhesion molecules are located on the cell surface and involved in the binding of other cells or extracellular matrix. Cell–cell and cell–matrix

interactions play vital roles in inflammation. In leukocytes and platelets, cell adhesion molecules interact with each other or with the extracellular matrix to bring circulating cells to areas of inflammation (Arnaout, 1993). Tissue- and inflammation-specific leukocyte/endothelial cell adhesion molecules constitute attractive targets for suppression or manipulation of the early stages of tissue inflammation (Jutila et al., 1989). To evaluate the inhibitory effects of SK compounds on cell adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin, HUVEC cultures were treated with SK compounds. The VCAM-1 and E-selectin mRNA expression was down-regulated in the 0.1–10 μM treated groups by reverse transcriptase PCR and real time PCR, while ICAM-1 expression was not affected by treatment with SK compounds (Fig. 2A).

3.2. Protein levels of adhesion molecules were inhibited by SK compounds

To confirm the inhibitory effect of SK compounds at the protein level, HUVEC were treated with SK compounds and stimulated with TNFα for 12 h. The levels of adhesion molecules were measured in conditioned media by ELISA. The comparative expression of ICAM-1 was 882.0 ± 16.1% in the TNFα treated group, 798.7 ± 11.7% in the combined TNFα and SK94 treated group, and 675.0 ± 16.7% in the combined TNFα and SK126 treated group when compared to the untreated control group (100.0%) (control vs. TNFα, TNFα vs. TNFα + SK94, TNFα vs. TNFα + SK126, all P values are <0.01). The VCAM-1 expression was 771.3 ± 14.3% in the TNFα treated group, 211.3 ± 11.1% in the combined TNFα and SK94 treated group, and 223.7 ± 6.1% in the combined TNFα and SK126 treated group when compared to the untreated control group (100.0%) (control vs. TNFα, TNFα vs. TNFα + SK94, TNFα vs. TNFα + SK126, all P values <0.001). E-selectin expression was 169.3 ± 8.2% in the TNFα treated group, 100.3 ± 7.8% in the combined TNFα and SK94 treated group, and 101.7 ± 11.1% in the combined TNFα and SK126 treated group when compared to the untreated control group (100.0%) (control vs. TNFα, TNFα vs. TNFα + SK94, TNFα vs. TNFα + SK126, all P values are <0.002) (Fig. 2B). Additionally, SK compounds were found to suppress the protein expression of ICAM-1, VCAM-1, and E-selectin in *in vitro* TNFα stimulated HUVEC cultures.

3.3. SK-compounds inhibit TNFα production in RAW 264.7 cells, Balb/c mice, and Behcet's disease mice

RAW 264.7 cells were treated with SK94 (1–70 μM) or SK126 (1–8 μM) for 30 min, after which they were stimulated with LPS for 18 h. The expression of TNFα was then quantified in conditioned media by ELISA. SK94 inhibited TNFα expression in cultures of RAW 264.7 cells in a dose

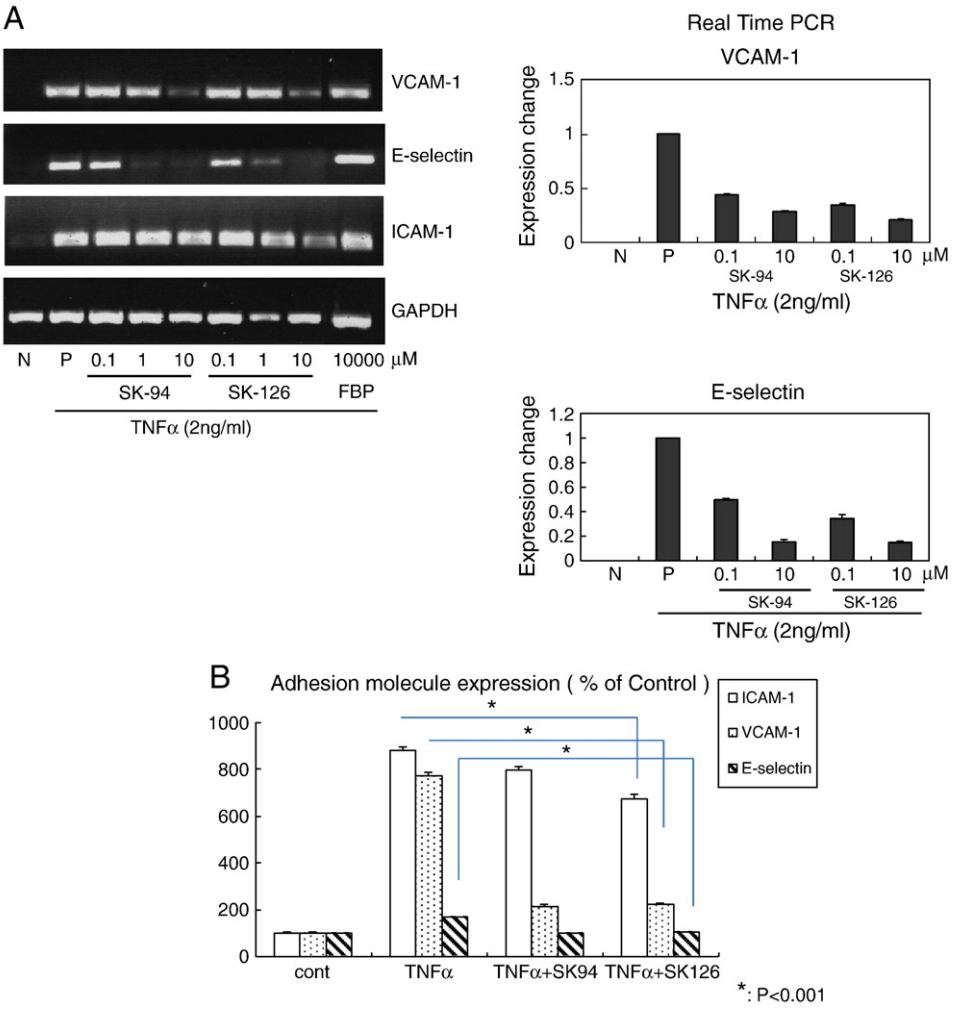

Fig. 2. A. mRNA expression of adhesion molecules inhibited by SK compounds. HUVEC were treated with SK compounds for 30 min and then stimulated with TNFα for 4 h. Cells were harvested, after which the RNA was extracted and applied to RT-PCR and real time PCR. N: negative control, P: positive control, FBP (far-upstream element binding protein): experimental control. B. Adhesion protein expression was inhibited by the effects of SK compounds in endothelial cells. Endothelial cells (HUVEC) were treated with SK compounds and stimulated with TNFα for 12 h. The quantities of adhesion molecules were determined by ELISA.

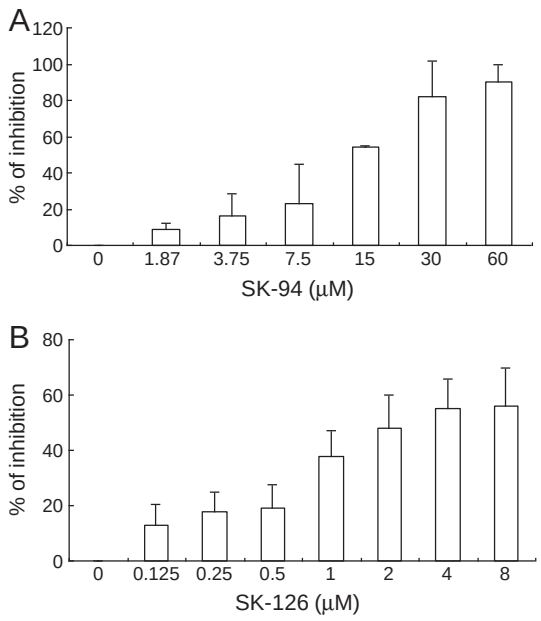

Fig. 3. SK compounds inhibit TNFα production in the RAW 264.7 cell line. SK94 (A) or SK126 (B) was applied to the cultures of RAW 264.7 cells for 30 min, after which they were stimulated for 18 h with LPS to express TNFα. The expression of TNFα was quantified by ELISA.

dependent manner. SK126 also inhibited TNFα expression more efficiently than SK94 at lower concentrations (Fig. 3). To determine if the SK compounds down-regulated the TNFα levels in serum, normal BALB/c mice were orally administered SK94 (1–70 mg/kg) or SK126 (0.1–100 mg/kg) (Table 1) (n = 8 per dose). SK94 and SK126 down-regulated the level of TNFα in the serum of Balb/c mice. SK94 (1 mg/mouse) or SK126 (1 mg/mouse) was also orally administered to Behcet's disease mice for five consecutive days to determine if it down-regulated the serum level of TNFα. At 4 h after the last treatment, the sera were collected and analyzed by ELISA. SK126 down-regulated TNFα (11.1 ± 13.4 pg/ml) when compared to the saline administered control (49.2 ± 9.2 pg/ml) (P = 0.01) (n = 5 per group). In addition, SK126 more efficiently down-regulated TNFα when compared to the thalidomide administered group (24.6 ± 13.9 pg/ml) (P = 0.02). Thalidomide is highly effective at suppressing oral and genital manifestations of Behcet's disease patients

Table 1
The inhibition of TNFα expression by oral administration of SK compounds in LPS challenged Balb/c mice. Balb/c mice were administered with SK94 (1–70 mg/kg) (A) or SK126 (0.1–100 mg/kg) (B). Two hours after the administration, mice were challenged intraperitoneally with LPS (1 mg/kg). Two hours after the LPS challenge, blood was collected and the sera were subjected to ELISA (n = 8 per dose).

Compound	Dose (mg/kg)	Inhibition (%)	ED_{50}
A.			
SK-94	70	84	14.2 mg/kg
	50	64	
	30	55	
	10	40	
	3	28	
	1	16	
B.			
SK-126	100	82	0.48 mg/kg
	10	78	
	3	71	
	1	66	
	0.3	55	
	0.1	22	

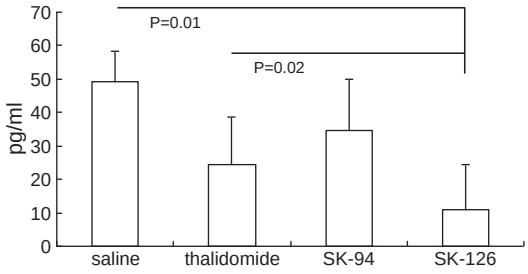

Fig. 4. SK compounds inhibit TNFα production in Behcet's disease mice. SK94 (1 mg/mouse) or SK126 (1 mg/mouse) was orally administered to Behcet's disease mice for five consecutive days. At 4 h after the last administration, the sera were collected and analyzed by ELISA.

(Bang, 1997) and Behcet's disease mice (Lee et al., 2004); however, SK126 more effectively down-regulated the serum level of TNFα than thalidomide in Behcet's disease mice (Fig. 4).

3.4. SK compounds down-regulate adhesion molecules in Behcet's disease mice

SK94 (1 mg/mouse) or SK126 (1 mg/mouse) was orally administered to Behcet's disease mice for five consecutive days to determine if it down-regulated soluble adhesion molecules (n = 7 per group). Soluble forms of ICAM-1, VCAM-1 and E-selectin are elevated during inflammatory conditions (Miles et al., 2001); however, SK126 significantly down-regulated soluble ICAM-1 in the sera of Behcet's disease mice (36.4 ± 5.6 ng/ml) when compared to the control (55.7 ± 17.6 ng/ml) (P<0.05). SK94 also down-regulated the soluble E-selectin (1048 ± 56 pg/ml) when compared to the control (1981 ± 221 pg/ml) (P<0.05) (Table 2). In spleen tissues, SK126 significantly down-regulated the soluble E-selectin (372 ± 397 pg/ml) when compared to the control (1100 ± 727 pg/ml) (P<0.05). Monocyte chemoattractant protein-1 (MCP-1) is an essential chemokine involved in monocyte traffic across endo- and epithelial barriers both *in vitro* and in vivo (Maus et al., 2002). MCP-1 also triggers firm adhesion of monocytes to vascular endothelium (Gerszten et al., 1999). SK126 inhibited MCP-1 (52.0 ± 12.3 pg/ml) when compared to the control (91.9 ± 73.0 pg/ml), but this inhibition was not statistically significant (Table 3).

3.5. SK compounds improved Behcet's disease mice

SK94 (1 mg/mouse) or SK126 (1 mg/mouse) was orally administered to Behcet's disease mice for five consecutive days to determine if they improved Behcet's disease symptoms. Behcet's disease symptoms were ameliorated after SK94 or SK126 administration. Specifically, SK94 improved the symptoms of Behcet's disease in five out of six mice (83%) and six out of seven mice (86%) in the SK126 group (P<0.005), while improvements only occurred in one of nine (11%) mice in the pH

Table 2
SK compounds down-regulated adhesion molecules in the sera of BD mice. SK compounds were orally administered to BD mice for 5 consecutive days, and then sera were obtained from each mouse and subjected to ELISA (n = 7).

Groups	Saline 100 μL/mouse	SK-94 1 mg/mouse (inhibition %)	SK-126 1 mg/mouse (inhibition %)	Thalidomide 50 μg/mouse (inhibition %)
Soluble ICAM-1 (ng/ml)	55.7 ± 17.6	42.5 ± 7.3 (23.8%)	36.4 ± 5.6[a] (34.6%)	47.8 ± 9.5 (14.2%)
Soluble VCAM-1 (ng/ml)	46.7 ± 8.4	43.1 ± 3.7 (7.8%)	40.2 ± 4.3 (14.1%)	43.2 ± 3.5 (7.6%)
Soluble E-selectin (pg/ml)	1981 ± 221	1048 ± 56[a] (47.1%)	1182 ± 669 (40.3%)	1843 ± 708 (6.9%)

[a] P<0.05.

Table 3
SK compounds down-regulated adhesion molecules, cytokine receptor, and chemokine in the spleen tissue of BD mice. SK compounds were orally administered to BD mice for 5 consecutive days, and then spleen tissues were obtained from each mouse and homogenized, and supernatant was subjected to ELISA (n = 7).

Groups	Saline 100 μL/mouse	SK-94 1 mg/mouse (inhibition %)	SK-126 1 mg/mouse (inhibition %)	Thalidomide 50 μg/mouse (inhibition %)
Soluble ICAM-1 (ng/ml)	608 ± 140	622 ± 221	637 ± 118	575 ± 142
Soluble VCAM-1 (ng/ml)	423 ± 115	469 ± 200	590 ± 192	477 ± 192
Soluble E-selectin (pg/ml)	1100 ± 727	1226 ± 1295	372 ± 397[a] (66.2%)	973 ± 764
Soluble TNFR1 (pg/ml)	2277 ± 121	2500 ± 721	2217 ± 1260	2012 ± 475
MCP-1 (pg/ml)	91.9 ± 73.0	69.6 ± 28.7 (24.3%)	52.0 ± 12.3 (43.4%)	81.5 ± 11.6 (11.3%)

[a] $P<0.05$.

1.2 saline (artificial gastric juice) group ($P<0.005$) and four of ten (40%) mice in the thalidomide group ($P<0.05$). The disappearance of symptoms or a decrease in the lesion size of more than 20% was classified as improvement. Photographs of the mice taken before and three weeks after administration were also compared (Fig. 5A), as was the severity score (Fig. 5B). In the SK126 treated group, the severity score was decreased from 2.56 ± 0.5 to 1.63 ± 1.02 ($P = 0.03$). In SK94 and thalidomide treated Behcet's disease mice the severity decreased from 2.17 ± 0.35 to 1.5 ± 0.76 and 2.61 ± 0.86 to 2.05 ± 1.47, respectively, but this change was not statistically significant. Conversely, the severity score in the saline injected group increased from 2.14 to 2.98.

4. Discussion

The results of the present study showed that synthesized pyridine compound derivatives can down-regulate the expression of TNFα and adhesion molecules in HUVEC and normal mice. Furthermore, these compounds down-regulated TNFα and adhesion molecules in Behcet's disease mice and ameliorated the symptoms. TNFα and adhesion molecules have been shown to be up-regulated in Behcet's disease patients (Sayinalp et al., 1996; Kose et al., 2008). In addition, down-regulation of TNFα using anti-TNFα antibody (Infliximab) was found to suppress various Behcet's disease symptoms including sight-threatening panuveitis (Sfikakis et al., 2001) and intestinal ulcers (Ju et al., 2007) in patients and mice (Choi et al., 2008). Another TNFα blocker, Etanercept, is also known to be an effective therapeutic agent for Behcet's disease patients and mice (Cantarini et al., 2009; Choi et al., 2008). All TNFα blockers are administered by injection; however, if orally administered TNFα blockers were available it would increase comfort for the patients. Orally administered SK compounds were found to effectively down-regulate the serum level of TNFα in LPS treated Balb/c mice and Behcet's disease mice. Additionally, SK126 more efficiently down-regulated the serum level of TNFα, soluble ICAM-1 (sICAM-1), and soluble E-selectin (sE-selectin) when compared to thalidomide in Behcet's disease mice. Thalidomide is known to be a TNFα inhibitor (Schmidt et al., 1996) that is effective in Behcet's disease patients (Hamuryudan et al., 1998) and Behcet's disease mice (Lee et al., 2004).

Infliximab treatment reduced the serum concentrations of soluble ICAM-1 and E-selectin in patients with juvenile idiopathic arthritis (Levälampi et al., 2007). In addition, subcutaneous injection of Etanercept down regulated the level of sICAM-1, sVCAM-1, and sE-selectin in the serum of rheumatoid arthritis patients (Klimiuk et al., 2009). SK126 and SK94 more efficiently down-regulated sICAM-1, sVCAM-1, and sE-selectin when compared to thalidomide in the sera of Behcet's disease mice, and it down-regulated sE-selectin when compared to the thalidomide treated group in the spleen tissues of Behcet's disease mice. SK126 and SK94 decreased monocyte chemotactic protein-1 (MCP-1) in the spleen tissues of Behcet's disease mice. MCP-1 has been found in the joints of people with rheumatoid arthritis, where it may serve to recruit macrophages and perpetuate inflammation (Harigai et al., 1993). Plasma MCP-1 levels have also been shown to be elevated in Behcet's disease patients (Kaburaki et al., 2003). In addition, MCP-1 inhibition ameliorated rat

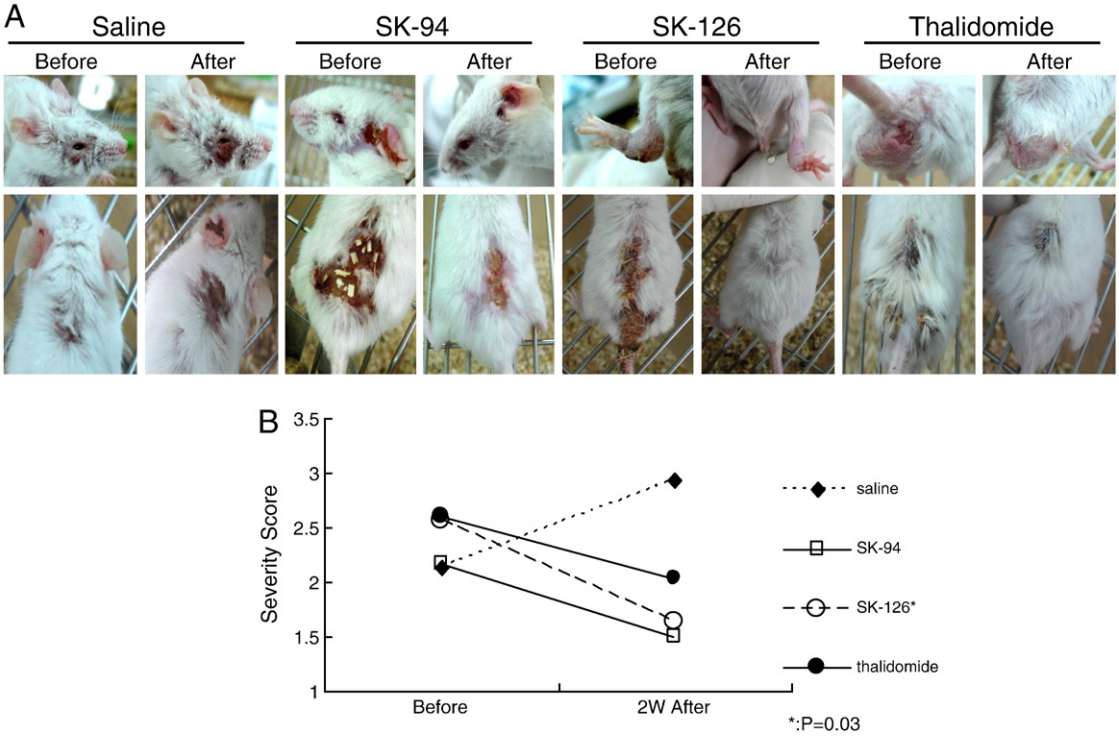

Fig. 5. Administration of SK compounds to Behcet's disease mice. A. Change in symptoms, B. change in severity score.

rheumatoid arthritis (Guglielmotti et al., 2002). Taken together, these findings indicate that the decrease in MCP-1 in response to treatment with SK compounds may contribute to the amelioration of Behcet's disease symptoms.

Thalidomide has been shown to effectively improve symptoms in Behcet's disease patients (Direskeneli et al., 2008) and mice (Lee et al., 2004). In the present study, the change in the severity score of SK94 treated Behcet's disease mice was similar to that of the thalidomide treated group. The decreasing inclination of severity score was steeper in the SK126 treated group than the thalidomide treated group, which demonstrates that SK 126 more effectively improves Behcet's disease symptoms than thalidomide. Thalidomide efficiently decreases TNFα and improves Behcet's disease symptoms, but it should not be prescribed during pregnancy. Overall, SK126 is more effective and safer than thalidomide and more convenient than Infliximab or Etanercept as a treatment for Behcet's disease.

In conclusion, SK compounds effectively down-regulated cell adhesion molecules at the mRNA and protein levels in HUVEC. SK compounds also inhibited TNFα in an *in vitro* culture of RAW 264.7 cells and in vivo in Balb/c mice. The down-regulated cell adhesion molecules and TNFα were correlated with the amelioration of Behcet's disease symptoms in mice. These findings suggest that SK compounds can be used as therapeutic agents to reduce the levels of TNFα and adhesion molecules during the treatment of inflammatory disease, especially through oral administration.

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