The Pharmacological Effect of Tangshan Spring Water On HaCaT Cells Treated With TNF- α

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Abstract

Background

Patients with psoriasis were respond to balneotherapy clinically. The mechanism remains uncertain.

Objective

To explore the pharmacological effect of Tangshan Spring Water (TSW) on HaCaT cell pre-treated with TNF- α .

Materials and Methods

A psoriasis-like model was constructed from HaCaT cell line by treatment with TNF- α for 48h *in vitro* and validated by determination of K17 expression. Then the modelled cells were treated with TSW and sodium hydrosulfide (NAHS), respectively. The MTT assay and flow cytometric assay were conducted to measure cell proliferation and apoptosis, respectively. The mRNA level of K17 was quantified using qPCR. The expression of proteins associated with MAPK pathway was quantified by Western blot assay.

Results

TNF- α treatment promoted HaCaT cell proliferation and increased K17 expression. Results of the MTT assay showed that TSW and NAHS inhibited the proliferation of HaCaT cells and abolished the effects of TNF- α . Moreover, TSW and NAHS induced cell apoptosis and decreased K17 mRNA levels in the model. Interestingly, the effect of NAHS was superior to that of TSW. Western blot assay results revealed that p-p38, p-ERK, and p-JNK levels were elevated by TNF- α treatment, especially the expression of p-p38. Both TSW and NAHS decreased the phosphorylation level of p38 but not JNK. Treatment with NAHS decreased p-ERK but not TSW treatment.

Conclusion

TSW inhibits proliferation and induces apoptosis in TNF- α -treated HaCaT cells. Treatment with TSW inhibits K17 expression via suppressing the activity of MAPK signaling pathway. These pharmacological effects may be attributed to the fact that NAHS is enriched in TSW.

Introduction

Psoriasis is a chronic inflammatory skin disease with a genetic background. It affects multiple systems and is associated with immune abnormalities. Conventional treatment options, such as topical use of

vitamin D3 derivatives, corticosteroids and systematic application of methotrexate, tretinoin, cyclosporin, as well as physical therapy represented by NB-UVB phototherapy, do not produce satisfactory results in most patients. Several novel treatment targets such as Tumor Necrosis Factor- α (TNF- α), interleukin-17 (IL-17), interleukin-23 (IL-23) have shown promising results in the treatment of psoriasis^{[1][2]}. However, high costs and side effects limit the application of such new drugs. These call for more affordable and safe strategies to complement existing therapies and enhance the patients' quality of life.

Balneotherapy, an ancient treatment of dermatosis involving bathing in thermal spring water, has been used for centuries in China and western countries. It is applied in the treatment of psoriasis with the advantages of cost, convenience and safety. The composition of spring water varies across regions, and so does its pharmacological mechanism.

Tangshan spring water (TSW), located in Jiangsu, China, is one of the sulfuric acid springs. The concentration of silicon, fluorine and strontium in the spring matches the standards for application in physiotherapy based on geothermal mineral water. TSW has been applied as a topical treatment in patients with psoriasis with good results in China. However, the anti-psoriasis mechanism of TSW is not clear. In the present study, we explored the effect of TSW on HaCaT cells treated by TNF- α .

Materials And Methods

1. Cell culture and major agents

HaCaT cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; PAN-Biotech, Aidenbach, Germany) under 5% CO₂ at 37 °C. TSW was collected from the NO.1 Water Well (Tangshan, Jiangsu, China) and stored in EP tubes at 4°C. NAHS was purchased from Sigma (USA) and diluted by distilled water.

2. MTT assay

After single treatment with TNF- α or co-treatment with TNF- α and TSW/NAHS, the viability of HaCaT cells in 96-well plates under different conditions was observed in line with the protocols of MTT assay. The absorbance of the cells was measured with a microplate reader at 450 nm.

3. RT-qPCR

Total RNA was extracted from HaCaT cells using TRIzol reagent (Takara, Japan). The RNA was reverse-transcribed into complementary DNA (cDNA) using PrimeScript RT reagent Kit (Takara, Japan). The RT-qPCR reaction was achieved using the SYBR Green PCR Kit (Sigma-Aldrich, USA). The relative mRNA level was calculated using the $2^{-\Delta\Delta Ct}$ method. In all the assays, PBS served as the loading control.

4. Measurement of cell apoptosis

Apoptosis level was assessed using the annexin V-FITC/PI apoptosis detection kit (BD Pharmingen, San Diego, CA, USA). Briefly, HaCaT cells were seeded into 6-well plates at a density of 4×10^5 cells per well, and cultured for 24 hours. They were then treated with TNF- α or co-treated with TNF- α and TSW or NAHS. After 24 hours, the cells were sampled and stained with annexin V and propidium iodide (PI). The sampled cells were resuspended in $1 \times$ binding buffer at a concentration of 1×10^6 cells/mL. Next, 100 μ L of the cell suspension was transferred into a 5-mL tube and mixed with 5 μ L of annexin V and 4 μ L of PI at room temperature for 15 minutes. Subsequently, 400 μ L of the $1 \times$ binding buffer was added to each tube and was analyzed using a flow cytometer within 1 hour.

5. Western-blot assay

Total proteins were extracted using the RIPA buffer (Thermo Fisher Scientific). The protein samples were resolved on 10% SDS-PAGE (Bio-Rad Laboratories, Hercules, CA, USA) and the nelectrotransferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membrane was then incubated with the following primary antibodies: anti-p38 (ab170099), anti-p-p38 (ab178867), anti-JNK (ab176645), anti-p-JNK (ab126424), anti-ERK (ab32537), anti-p-JNK (ab192591) purchased from Abcam (Cambridge, UK). Subsequently, the membrane was incubated with secondary antibodies. Relative protein expression level was determined using Image J software (NIH Image, Bethesda, MD, USA).

6. Statistical Analysis

Data from at least three independent experiments were presented as the mean $\pm$ standard deviation. All analyses were performed using the Prism 6.0 (GraphPad, San Diego, CA, USA). Difference between groups were compared using the Two-tailed Student's *t*-test or one-way ANOVA. $P < 0.05$ was considered the threshold of statistical significance.

Results

1. The effect of TNF- α on K17 expression and the proliferation in HaCaT cells

The results of MTT assay revealed that TNF- α promoted HaCaT cells proliferation in a concentration-dependent manner (Fig. 1A). Moreover, TNF- α at the concentration of 50nM up-regulated the expression of K17 markedly (Fig. 1B). The findings indicate the successful establishment of a psoriasis model in HaCaT cells.

2. The effect of TSW and NAHS on proliferation and apoptosis in TNF- α -treated HaCaT cells

NAHS has been found to be an important ingredient of TSW that participates in the pathogenesis of multiple skin diseases including psoriasis^[3]. In the present study, we disposed TNF- α -treated HaCaT cells with TSW and 400uM NAHS. Analysis of MTT assay results demonstrated that both TSW and NAHS abolished the pro-proliferation effects of TNF- α in HaCaT cells (Fig. 2A). Similarly, TSW and NAHS abolished the pro-apoptosis effects of HaCaT cells caused by TNF- α (Fig. 2B~F).

3. The effect of TSW and NAHS on K17 expression in TNF- α -treated HaCaT cells

To investigate the therapeutic effects of TSW and NAHS on psoriasis, we quantified the expression of K17, which is considered as a biomarker of psoriasis, in TNF- α -treated HaCaT cells by qPCR assay. The results showed that TSW or NAHS treatment decreased K17 expression significantly. Notably, the effect of NAHS was stronger than that of TSW as shown in Fig. 3.

4. The effect of TSW and NAHS on the activity of MAPK pathway in TNF- α -treated HaCaT cells

Previously, studies have demonstrated that the MAPK signaling pathway plays a critical role in the pathogenesis of psoriasis, particularly cooperate with the receptor of TNF- α . Thus, we suspect that the MAPK signaling may contribute to the anti-psoriasis effect of TSW and NAHS. Accordingly, the activity and level of major proteins involved in the MAPK signaling pathway have been quantified by Western blotting assay. The results showed that the phosphorylation level of p38 (p-p38), ERK (p-ERK), and JNK (p-JNK) was significantly increased after treated by TNF- α . Interestingly, treatment with TSW and NAHS abolished the effect of TNF- α on p-p38 in HaCaT cells. Moreover, NAHS showed stronger effects compared to TSW. On the other hand, NAHS inhibited the expression of p-ERK but not p-JNK (Fig. 4).

Discussion

Numerous studies have shown that immune imbalance and systemic inflammation contribute to the incidence and pathogenesis of psoriasis. Skin-infiltrating inflammatory cells, especially T-lymphocytes, promote keratinocyte over-proliferation and keratinization by secreting multiple inflammatory factors, including TNF- α ^[4]. Recently, the TNF- α /IL23/IL17A signaling pathway was identified as a critical driver of psoriasis pathogenesis. It regulates keratinocytes, dendritic cells (DCS), T cells and neutrophils, and forms multiple feed-loop pathways in a cross-talk manner among these cells^[5].

Many immune-targeted agents have been used in the treatment of psoriasis patients and achieved good results. So far, five agents targeting TNF- α or its receptor (etanercept, infliximab, adalimumab, cetuzumab, and golimumab) have been approved by FDA for the management of psoriasis^[6–14].

The MAPK signaling pathway, which is a downstream signaling pathway of TNF- α receptor, regulates cell proliferation, apoptosis, and differentiation, thereby influencing the pathogenesis of psoriasis. Three kinases, p38, JNK, and ERK1/2, regulate the activity of the MAPK pathway. Previously, a high level of phosphorylated p38 (p-p38) was detected in psoriasis lesions, indicating that the MAPK signaling pathway may be involved in the incidence of psoriasis. The MAPK signaling pathway also regulates levels of multiple inflammatory factors^[15].

Tangshan hot spring is the only hot spring in China that has obtained the international double certification of both European and Japanese. TSW, as a nature agonist of TNF- α signaling pathway in psoriasis model, could be a complementary and adjuvant therapy of therapy. In the present study, treatment with TNF- α increased the phosphorylation of p38, ERK, and JNK in HaCaT cells which

promoted the viability and K17 expression in the cells. However, these effects were abolished following co-treatment with TSW or NAHS (a component of TSW). These findings are consistent with previous reports. It has reported that TNF- α promoted the phosphorylation of kinases in the MAPK pathway in HaCaT cells. Another study showed that H₂S can treat psoriasis based on *in vitro* models^[16]. These results imply that TSW prevents psoriasis by regulating the activity of the MAPK pathway, especially p-p38. TSW-derived NaHS may be the critical active ingredient.

This study has the following limitations. First, we only investigated the role of TNF- α in psoriasis pathogenesis although there are multiple factors involved in its development. Secondly, the study is based on *in vitro* experiments thus *in vivo* models should be employed in future to validate the present results. Lastly, although there are multiple components in TSW, we only focused on NAHS in this study.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

Authors declared no competing interests.

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Authors' contributions

TC & GS designed the experiments. SY & ZX wrote the manuscript, conducted experiment. CF & LJ executed the statistical analysis. SMJ reviewed the manuscript.

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Figures

Figure 1

HaCaT cells treated with TNF-α for 24h. A.proliferation detected by MTT assay; B.K17 expression detected by qPCR.*P<0.05 VS 0nM;**P<0.01 VS 0nM.

Figure 2

Cellular proliferation and apoptosis affected by different treatments. A. cell proliferation detected by MTT assay; B~F. cellular apoptosis confirmed with V-FITC/PI stain; B. apoptosis rate in each group; C. control; D. TNF- α ; E. TNF- α +TSW; F. TNF- α +NASH; *P<0.05 vs TNF- α ; **P<0.01 vs TNF- α ; #P<0.05 vs. TNF- α +TSW

Figure 3

K17 mRNA levels in HaCaT cells after variety treatments. ** $P < 0.01$ vs. TNF- α ; # $P < 0.05$ vs. TNF- α +TSW

Figure 4

Critical proteins in MAPK signaling pathway detected by Western-blot assay. * $P < 0.05$ vs TNF- α ; ** $P < 0.01$ vs TNF- α ; # $P < 0.01$ vs TNF- α +TSW.