



Original Article



The combination of tetramethylpyrazine and vitamin A acid in the treatment of skin photoaging by activating the HIF-1 α pathway

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Abstract



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Skin photoaging is a skin degenerative disease that causes patients to develop malignant tumors. The existing clinical treatment of photoaging has limitations. This greatly reduces the recovery rate of photoaging patients. Studies have confirmed that *Ligusticum wallichii* Franch (LWF) monomer tetramethylpyrazine (TMP) alleviates various skin diseases. The combination of traditional Chinese medicine and Western medicine helps with this process. Our research aimed to explore the specific treatment mode and molecular mechanism of TMP in treating skin photoaging. CCK-8 assays were used to evaluate the activity and toxicity of HaCaT cells. β -galactosidase aging, Carbonyl compound and nitrosylated tyrosine assays were used to analyze the aging of HaCaT cells. ROS assays and ELISA were used to analyze the enrichment of ROS. The molecular docking experiment analyzed the binding of TMP and HIF-1 α . qRT-PCR and Western blot were used to detect the activation of skin aging-related pathways. HE staining was used to analyze the thickness of the stratum corneum skin on the back skin of mice. 200 μ g/L LWF alleviates cellular photoaging and mouse skin photoaging by reducing ROS enrichment. Its monomer TMP plays an important role in this process. The combination of TMP and HIF-1 α accelerates the degradation of ROS by activating the Nrf2/ARE signaling pathway. This process reduces the apoptosis of cells damaged by light. In addition, we also found that the combination of TMP and retinoic acid (RA) is more beneficial for the treatment of skin damage caused by light in mice. The combination therapy of TMP and RA alleviates skin oxidative stress response through overexpression of HIF-1 α . This plan is beneficial for the treatment of skin photoaging.

Keywords: HIF-1 α , *Ligusticum wallichii* Franch, Retinoic acid, ROS, Skin photoaging, Tetramethylpyrazine.

1. Introduction

Due to the intensification of aging worldwide, skin aging is gradually receiving widespread attention. Among them, the incidence rate of skin photoaging is about 85% [1]. It often occurs in parts of the human body exposed to ultraviolet (UV) rays of sunlight, such as hands, face, and forearms. This causes pigmentation, capillary dilation, rough skin, and the formation of wrinkles. More severe photoaging leads to skin cancer in patients. This type of cancer is highly susceptible to metastasis and spread, ultimately endangering the lives of patients [2]. The current treatment methods mainly include medication, chemical exfoliation, and laser therapy. Among them, drug therapy has a low risk and a high safety factor. However, existing drug therapies such as retinoic acid (RA) and antioxidants have high limitations [3]. Therefore, finding new anti-photoaging treatment plans is quite important in clinical practice.

Excessive exposure to UV usually leads to excessive accumulation of Reactive Oxygen Specifications (ROS) in cells, also known as photodamage to cells. Excessive ROS in the body's skin leads to oxidative stress, inflam-

matory reactions, collagen degradation, cell apoptosis, and decreased water content [4]. They severely damage the tissue structure and function of the skin, such as changes in epidermal thickness, accelerated degradation of collagen and elastic fibers, and microvascular dilation [5]. Traditional Chinese Medicine (TCM) has been proven to effectively inhibit the excessive accumulation of ROS in various diseases, such as Aconite, Baihe Gujin Pill, and Modified Shoutai Pill [6–8]. The active monomers of TMC interact with drug targets in the patient's body. Common targets include biological macromolecules such as receptors, enzymes, ion channels, and transporters. Numerous clinical trials have confirmed that a reasonable combination of TCM monomers and Western medicine can shorten the course of various diseases and improve the cure rate of patients, such as chronic obstructive pulmonary disease, urticaria, and heart disease [9]. *Ligusticum wallichii* Franch (LWF) is considered a TCM beneficial for promoting blood circulation and resolving stasis, and it is also one of the common drugs used in combination with Western medicine to assist in the treatment of diseases [10]. Tetramethylpyrazine (TMP) is the most important monomer

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in LWF. However, the therapeutic modes and molecular pathways of LWF and TMP for photoaging skin have not been fully explored.

Hypoxia-inducible factor-1 α (HIF-1 α) belongs to the basic helix loop helix Per Arnt Sim family of transcription factors and is also an active subunit of HIF. The end of HIF-1 α is the regulatory region for oxygen signal activity, indicating that it is regulated by oxygen signal concentration [11]. Existing research has confirmed that the HIF-1 α pathway is activated under hypoxic conditions. This effectively reduces the consumption of oxygen and the generation of ROS. Under high oxygen conditions, the presence of high concentrations of ROS can also activate HIF-1 α . Therefore, this protein is often considered beneficial for the regulation of oxidative stress response and the normal transport of intracellular oxygen [12]. However, the role of HIF-1 α in the treatment of skin photoaging by TCM is not yet clear.

Our work aims to explore the specific molecular mechanisms and medication patterns of TMP in the treatment of skin photoaging. Firstly, we provide evidence that LWF and its monomer TMP can alleviate cellular photodamage and photoaging of mice skin, and this process is related to the HIF-1 α pathway. Finally, the results of in vivo and in vitro experiments confirmed that the binding of TMP and RA alleviates oxidative stress response through overexpression of HIF-1 α . This plan is beneficial for the treatment of skin photoaging.

2. Materials and methods

2.1. Clinical sample collection

We recruited 12 women (age: 52.4 \pm 1.5) affected by facial photoaging and 12 healthy controls (age: 49.2 \pm 1.2 years old). We collected 5 mL of blood samples from the subjects. They signed an informed consent form and understood the research purpose in a conscious state. This study was approved by the Clinical Research Ethics Review Committee of Guangdong Provincial Hospital of Chinese Medicine.

2.2. Cell culture

HaCaT cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. Cells were cultured under the same standard conditions (5% CO₂ and 37°C). Cells were cultured in DMEM complete culture medium (DMEM base medium: total body serum: penicillin/streptomycin=89:10:1). When the cell grows to around 85% convergence, 0.25% (w/v) trypsin solution is used for cell passage.

2.3. Establishment of a cell model for light damage

HaCaT cells were cultured on a 12-well plate for 24 hours, and the culture medium was removed and washed. Place the cells under a 31.2 mJ/cm² UVB (310 nm) ultraviolet lamp for 8 minutes. After washing the cells, continue incubation with DMEM medium for 24 hours. Then, we constructed a drug-induced photo injury cell model by replacing the culture medium containing RA (0.01 mg/mL), different concentrations of LWF (0, 100, 150, 200, 300 and 500 μ g/L), and TMP (0, 40, 80 and 160 μ g/L) [13].

2.4. Cell transfection

Small interfering RNA HIF-1 α (sh-HIF-1 α) and its

negative control (sh-NC) were purchased from GenePharma (Shanghai, China). Transfections were performed using Lipofectamine 2000 reagent (Invitrogen, California, USA), according to the manufacturer's instructions.

2.5. qRT-PCR

Cells and human serum were extracted total RNA by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The addition of gDNA Eraser (TaKaRa, Liaoning, China) facilitates the removal of genomic DNA. SuperScript IV (ThermoFisher) was used to reverse transcribe total RNA. One Step SYBR® PrimeScript RT-PCR Kit (TaKaRa) was used to perform qRT-PCR analysis on the ProFlex™ PCR system (ThermoFisher). PCR primers are listed following. HIF-1 α forward: GAACGTCGAAAAGAAAAGTCTCG. HIF-1 α reverse: CCTTATCAAGATGCGAACTCACA. β -actin forward: 3'-AGCGAGCATCCCCCAAAGTT-5'. β -actin reverse: 3'-GGGCACGAAGGCTCATCATT-5'. β -actin is the internal control.

2.6. Western blot

RIPA Buffer Concentrate (Cayman Chemical, State of Michigan, USA) was used to lys cells and mice tissues. The total protein was transferred to SDS-PAGE for constant pressure electrophoresis for 120 min. Isolated proteins were transferred to the Immobilon-E-PVDF membrane (Merck, Darmstadt, Germany). The membrane was incubated with primary antibodies at 4°C for 12 h. The secondary antibodies were incubated for 2h. Enhanced chemiluminescence was used to observe the proteins. The antibodies used in our work are shown below: anti-TNF α (ab307164, Abcam, Cambridge, UK), anti-IL1 β (ab216995, Abcam), anti-IL6 (ab259341, Abcam), anti-HIF1 α (ab308433, Abcam), anti-TGF β 1 (ab215715, Abcam), anti-Smad (ab13723, Abcam), anti-p-NF- κ B (ab239882, Abcam), anti-NF- κ B (ab32360, Abcam), anti-NFATc1 (ab25916, Abcam), anti-Nrf2 (ab137550, Abcam), anti-Keap1 (ab227828, Abcam), anti-SOD1 (ab308181, Abcam), anti-CAT (ab209211, Abcam), anti-NQO1 (ab80588, Abcam), anti-ERK1 (ab32537, Abcam), anti-p-ERK1 (ab131438, Abcam), anti-p38 (ab31828, Abcam), anti-p-p38 (ab178867, Abcam) and β -actin (ab8226, Abcam).

2.7. CCK-8

According to the manufacturer's protocol, the transfected HaCaT cell lines were incubated with 10 μ L CCK-8 solution (Beyotime) in 100 μ L DMEM medium containing FBS. Absorbances were measured at 450 nm.

2.8. β -Galactosidase staining

The β -gal Aging Detection Kit (Bohe Biopharmaceutical Technology, Wuxi, China) was used to analyze the aging status of HaCaT cells. In short, after centrifugation, the cells were collected and washed, and 1 mL of Fixative Solution was added to fix at room temperature for 15 minutes. After washing the cell fixation solution, add the working solution and mix well. Incubate overnight at 37 °C and calculate the number of positive cells using an Olympus BX43 optical microscope (Tokyo, Japan).

2.9. ROS measurement

Reactive Oxygen Species Assay Kit (Beyotime, Shanghai, China) was used to detect ROS levels of blood and

intracellular samples. Intracellular total ROS levels were measured using 2',7'-dichlorofluorescein diacetate (Beyotime, Shanghai, China) according to the manufacturer's instructions. Subsequently, fluorescence emission peaks were detected using BD FACSCalibur (BD Biosciences, New Jersey, USA).

2.10. Enzyme-linked immunosorbent assay (ELISA)

The SOD, CAT and GSH-PX ELISA kits were purchased from Biovision (California, USA). The experiment was carried out according to the manufacturer's instructions.

2.11. Carbonyl compound and nitrosylated tyrosine

Protein carbonyl content assay kits (Abcam, ab126287) and protein carbonyl compound assay kits (ELK Biotechnology, Wuhan, China) were used to assess cells oxidative damage. The experiment was carried out according to the manufacturer's instructions.

2.12. Hematoxylin-eosin staining (HE staining)

The mice skin tissues were fixed using Hematoxylin-Eosin staining (Klamar, China, Shanghai), and paraffin was embedded and sliced into 5µm. Hematoxylin and eosin were used to stain sections. BX51 optical microscope was used to observe skin thickness of the stratum corneum.

2.13. Light damaged mice

SD mice were purchased from the National Rodent Seed Center (Shanghai, China). Mice weights are ≥200 and <250 g. Mice were fed in a feeding box at 25°C with humidity was 50-60%. The light and dark cycle time of the feeding box was 12:12 hours. Mice freely obtained feed and water. All procedures involved were strictly approved by the Animal Protection and Utilization Committee of Guangdong Provincial Hospital of Chinese Medicine and performed following the Animals (Scientific Procedures) Act 1986.

Randomly divide mice into 6 groups (n=8): Control group (Con), UVB group (UVB: 0.07J/cm²), UVB+0µg/L TMP+RA group, UVB+100µg/L TMP+RA, UVB+200µg/L TMP+RA and UVB+300µg/L TMP+RA. Electric shavers were used to shave off the back hair of mice. Mice were irradiated with UVB (0.07 J/cm²) ultraviolet light five times a week for 9 weeks. Before irradiation, RA gel was used to smear mouse skin or TMP solution was used to inject into the mouse peritoneum. After the process was completed, the mice were anesthetized. The Tissue Viability Imager TiVi 700 (WheelsBridge, Sweden) was used to take photos and analyze the melanin content of mice. Finally, the mice were executed. The back skin of mice was used for subsequent analysis [14].

2.14. Bioinformatics analysis

GSE41078 dataset was obtained from the GEO database to assess the expression patterns of skin photoaging-related differentially expressed genes using R language. The TCMSP database (<https://tcmsp.com/tcmsp.php>) retrieves the effective active compounds and corresponding targets of LWF. The Bioinformatics & Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) drew the Venn diagram of down-regulated genes from the GSE41078 and corresponding targets of LWF.

TMP molecular structure from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Chem3D software is used for format conversion and energy minimization. Schrodinger software is used to establish databases through hydrogenation, structural optimization, and energy minimization. The processed TMP molecular structure serves as the ligand molecular database for molecular docking. HIF-1α protein structure from RCSB database (<https://www.rcsb.org/>). The protein structure was processed on the Maestro 11.9 platform using Schrodinger's Protein Preparation Wizard. The ligands and receptors for molecular docking are screened and aligned using the Standard Precision (SP) method using Schrödinger Maestro software. Finally, PyMol12.1 visualization software was used to visualize the structural domain of the interaction between TMP and HIF-1α.

2.15. Statistical analyses

The SPSS16.0 software (Chicago, USA) was used to conduct data analysis. Data were presented as mean ± standard deviation from at least three independent replicates. Statistical differences between groups were calculated using one-way ANOVA. Differences were considered statistically significant at p<0.05.

3. Results

3.1. LWF alleviates light damage to HaCaT cells and skin photoaging in mice

To investigate the effect of LWF on skin photoaging, we analyzed the effects of LWF on cells and mouse models. As shown in Figure 1A, 200 µL WF at g/L has the lowest toxicity to cells. The changes in TNF-α, IL-1β and IL-6 proteins also indicate that the addition of LWF alleviates the inhibition of cell growth by UVB (Figure 1B). Compared with the UVB group, the LWF+UVB group had higher ROS content, SOD, CAT, and GSH-PX enzyme activity (Figures 1C, D). Compared with the UVB group, the LWF+UVB group had a higher content of carbonyl com-

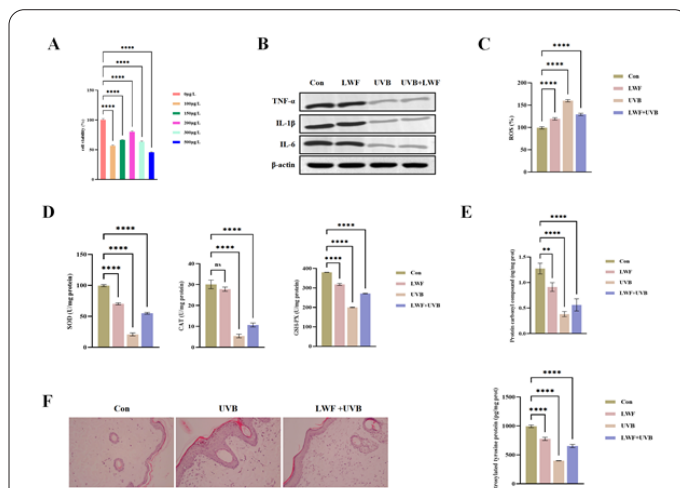


Fig. 1. LWF alleviates photoaging in HaCaT cells and mice. (A) CCK8 method for detecting cell viability. (B) Changes in protein expression of TNF-α, IL-1 β, and IL-6 in cells. (C) Detection of cellular reactive oxygen species (ROS) levels. (D) Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) activity detection in cells. (E) Effect of LWF on the levels of protein carbonyl compound and nitrosylated tyrosine protein in HaCaT cells. (F) HE staining was used to evaluate the issue structure of mouse skin. ***p<0.0001.

pound and nitrosylated tyrosine (Figure 1E). On the other hand, the thickness of the stratum corneum in Con and LWF+UVB mice was lower than that in the UVB group (Figure 1F). The above results confirm that LWF alleviates the process of skin photoaging both in vivo and in vitro.

3.2. Pharmacological analysis of LWF in the treatment of photoaging

To explore the specific mechanism of LWF in treating skin photoaging, we analyzed the active components of LWF. Firstly, we searched the TCMSP database for the effective active compounds and their corresponding targets of LWF. Based on previous research, we speculate that TMP plays an important role in this process (Figure 2A) [15]. As shown in Figure 2B, we screened differentially expressed genes in the GSE41078 dataset. Secondly, we found through Venn diagram analysis that there are three genes in the cross set between the GSE41078 dataset and the TMP target (Figure 2C). Next, we analyzed the expression of these three genes in photoaging patients through qRT-PCR. The results showed that both HIF-1 α and VEGFC were highly expressed. Among them, the expression of HIF-1 α is more significant (Figure 2D). Finally, we found an interaction between HIF-1 α and TMP through molecular docking analysis (Figure 2E). These results indicate that TMP is involved in the progression of skin photoaging by regulating the expression of HIF-1 α .

3.3. TMP alleviates cell light damage by promoting the expression of HIF-1 α

To explore the specific ways in which TMP alleviates photoaging, we constructed a photoinduced cell model. As shown in Figure 3A, the enrichment of HIF-1 α in the UVB+TMP group is higher than that in the Con and UVB groups. The results of CCK-8 showed that the cell viability of the UVB group and UVB+TMP+sh-HIF-1 α group was significantly lower than that of the Con group and UVB+TMP group (Figure 3B). The results of flow cytometry apoptosis detection showed that the apoptosis rate of cells in the UVB group and UVB+TMP+sh-HIF-1 α group was significantly higher than that in the Con group and UVB+TMP group (Figure 3C). The results of Western blot showed that the levels of TNF- α , IL-1 β and IL-6 protein enrichment in cells of the UVB group and UVB+TMP+sh-HIF-1 α group were lower than those of the Con group and UVB+TMP group (Figure 3D). These results confirm that the addition of TMP reduces cell light damage through HIF-1 α .

3.4. TMP and RA jointly alleviate light damage in HaCaT cells

To investigate the effect of combined use of TMP and RA on skin photoaging, we investigated the effects of different concentrations of TMP and RA combined use on photodamaged cells. The results of CCK-8 analysis and Western blot detection showed that compared to 80 μ g/L TMP+RA group, the cell viability and proliferation rate in RA group, 40 μ g/L TMP+RA group, and 160 μ g/L TMP+RA group were lower (Figures 4A, B). Then, the results of ELISA and ROS content detection showed that compared to 80 μ g/L TMP+RA group, ROS accumulation, SOD, CAT, and GSH-PX enzyme activity in RA group, 40 μ g/L TMP+RA group, and 160 μ g/L TMP+RA group were lower (Figures 4C, D). Finally, we constructed light-

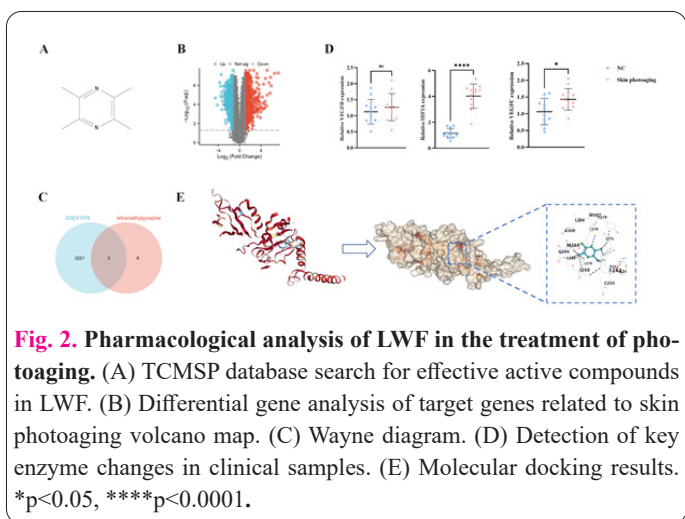


Fig. 2. Pharmacological analysis of LWF in the treatment of photoaging. (A) TCMSP database search for effective active compounds in LWF. (B) Differential gene analysis of target genes related to skin photoaging volcano map. (C) Venn diagram. (D) Detection of key enzyme changes in clinical samples. (E) Molecular docking results. * $p < 0.05$, *** $p < 0.0001$.

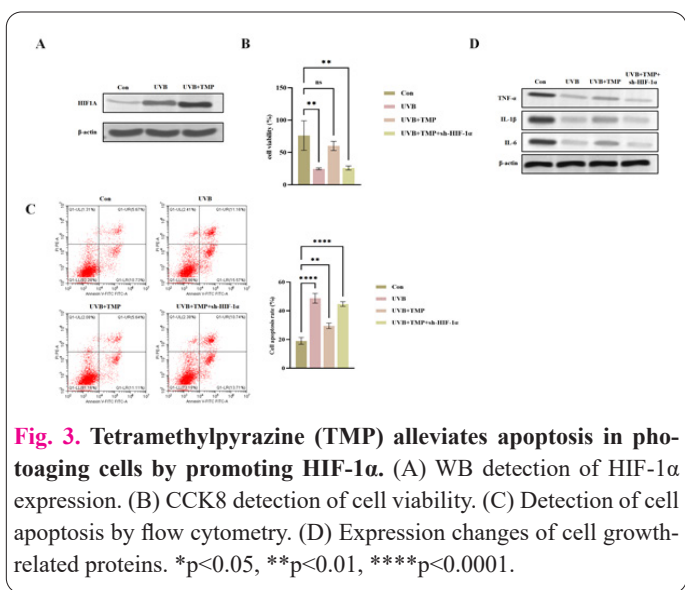


Fig. 3. Tetramethylpyrazine (TMP) alleviates apoptosis in photoaging cells by promoting HIF-1 α . (A) WB detection of HIF-1 α expression. (B) CCK8 detection of cell viability. (C) Detection of cell apoptosis by flow cytometry. (D) Expression changes of cell growth-related proteins. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

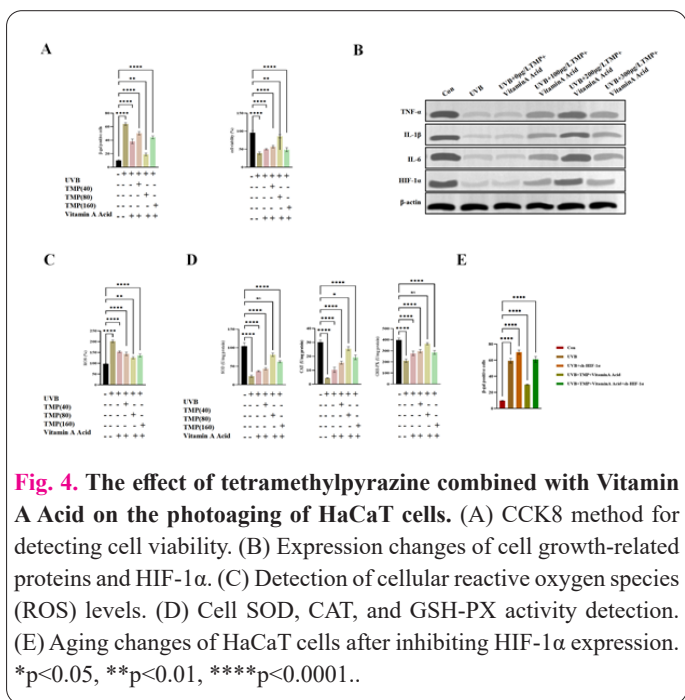


Fig. 4. The effect of tetramethylpyrazine combined with Vitamin A Acid on the photoaging of HaCaT cells. (A) CCK8 method for detecting cell viability. (B) Expression changes of cell growth-related proteins and HIF-1 α . (C) Detection of cellular reactive oxygen species (ROS) levels. (D) Cell SOD, CAT, and GSH-PX activity detection. (E) Aging changes of HaCaT cells after inhibiting HIF-1 α expression. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

damaged cells with low expression of HIF-1 α . The results of Senescent β -galactosidase analysis showed that inhibiting HIF-1 α expression increased the number of β -gal positive cells (Figure 4E). The above results confirm that the combined use of TMP and RA alleviates cell light damage

by promoting the expression of HIF-1 α .

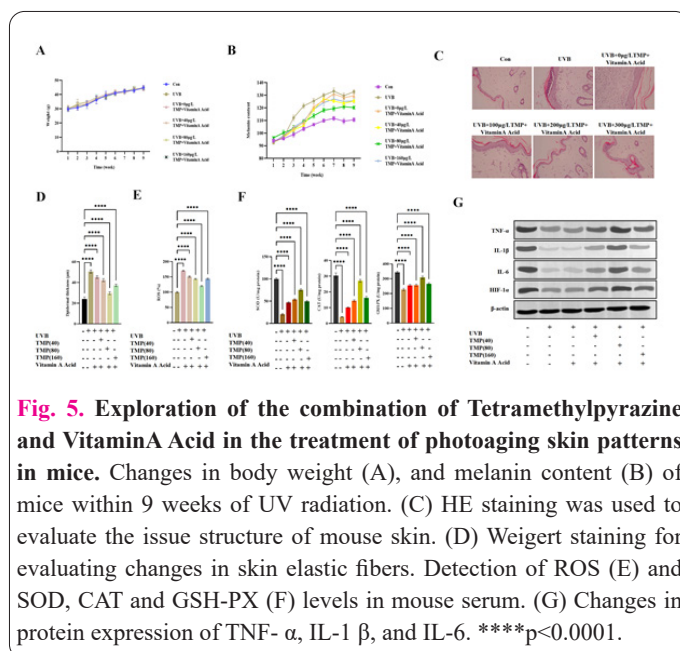
3.5. TMP and RA combined therapy for photoaging of mice skin

To investigate the effect of TMP combined with RA treatment in vitro, we constructed a photoaging mice model. We found that under the same body weight of mice, the skin melanin content of the 80g/L TMP+RA group and Con group mice was lower (Figures 5A, B). As shown in Figures 5C, and D, the epidermal thickness of the skin of mice in the 80 μ g/L TMP+RA group and Con group was higher. The results of ELISA showed that compared to 80 g/L TMP+RA group, ROS accumulation, SOD, CAT, and GSH-PX enzyme activity in RA group, 40g/L TMP+RA group, and 160 μ g/L TMP+RA group were lower (Figure 5E, F). Western blot analysis showed that the enrichment of growth-related proteins and HIF-1 α was higher in the skin of mice in the 0 μ g/L TMP+RA group and Con group (Figure 5G). In summary, the combination of 80 μ g/L TMP and RA is more beneficial for the treatment of photoaging in mice skin.

4. Discussion

In recent years, multiple clinical studies have confirmed that LWF plays a role in the treatment of various diseases, which mainly depends on its active ingredients [16]. Our research aims to explore the specific mechanisms and medication methods of LWF and its monomer TMP in the treatment of skin photoaging. We found through bioinformatics analysis and in vitro experiments that the combined effect of TMP and RA alleviates cell light damage by promoting the expression of HIF-1 α . In vivo experiments have confirmed that this medication regimen greatly alleviates skin photoaging in rats.

UV-induced photoaging is the main culprit of skin aging. The photoaging of the skin causes thickening of the stratum corneum, loss of collagen, inflammatory reactions, and cell apoptosis. This is mainly due to the excessive accumulation of ROS [17]. RA is currently the only *Food and Drug Administration* approved photoaging therapy drug [18]. However, multiple studies have confirmed that TCM is equally effective in treating these symptoms, such as bazi bushen, seawater pearl, and cordyceps cicadae [19, 20]. LWF is well known as an important TCM for assisting in the treatment of various diseases, such as heart and vascular diseases [21]. Our study confirms for the first time that LWF alleviates skin photoaging. The monomer TMP of LWF is considered one of the main active components that play a role in different diseases of LWF [22]. Jiang *et al.* [15] found that TMP can treat psoriasis. The study by Wu *et al.* [23] confirmed that TMP can inhibit the proliferation of skin fibroblasts. Similar to the above research, we also found that TMP has a promoting effect on the treatment of skin photoaging. At the same time, we also explored specific plans for TMP treatment. The combination of traditional Chinese and Western medicine has been proven to be more beneficial for the treatment of skin diseases. Alalaiwe *et al.* [24] found that the combination of burdock extract and Western medicine light therapy is more beneficial for the relief of psoriasis. Yu *et al.* [25] findings indicate that the addition of Ailantone is beneficial for the treatment of skin melanoma with anti-PD-L1. Interestingly, our study also found that the combination therapy of TMP and RA can effectively alleviate cellular



photoaging and mouse skin photoaging. Regarding the selection of drug concentration, we speculate that TMP follows an S-shaped pharmacodynamic curve during the treatment of photoaging. Although in our experiment, mice received TMP through solution injection, Zhao *et al.* [26] have shown that TMP infiltrates the body through different transdermal drug delivery systems. Therefore, our research has also laid the foundation for the future treatment strategies of TMP topical drugs.

HIF-1 α is a sensor used by the body to coordinate the external environment, which is often associated with oxidative stress response and ROS accumulation in the body. The defect of HIF-1 α has also been proven to be a key factor in skin aging [27]. Pagani *et al.* [28] found that HIF-1 α is involved in the regeneration of skin cells and tissues. Bonham *et al.* [29] also proposed that the expression of HIF-1 α is beneficial for alleviating UVB-induced damage to keratinocytes. Our study also validated that the expression of HIF-1 α promotes the anti-photoaging process of skin cells. The increased expression of HIF-1 α downstream gene VEGFC in clinical sample analysis also confirms our viewpoint. Nrf2/ARE is an anti-photoaging pathway associated with ROS clearance. Wen *et al.* [30] confirmed in their study that the Nrf2/ARE/HIF-1 α pathway promotes the healing of skin injuries. Our work also confirms that this pathway also promotes the relief of skin damage caused by light. Based on the analysis of ROS content, we believe that HIF-1 α reduces the accumulation of ROS in the skin through the Nrf2/ARE pathway, thereby reducing skin cell apoptosis. Therefore, the progression of mouse skin photoaging was alleviated. In addition, the expression changes of HIF-1 α are often important targets for traditional Chinese medicine in treating skin photoaging. Xu *et al.* [31] found that Haoqin Huaban formula promotes the expression of HIF-1 α and alleviates UVB induced skin damage. Yang *et al.* [32] found that physis pubescent alleviates skin inflammation caused by photoaging through HIF-1 α signaling. Our work confirms that TMP promotes the expression of HIF-1 α and alleviates skin photoaging. Liggins *et al.* [33] discovered that RA treats skin diseases through the HIF-1 α pathway. This provides us with ideas for our work. Our study further found that the combination of TMP and RA promotes the expression of HIF-1 α in the

treatment of skin photoaging.

5. Conclusion

In summary, our study groundbreaking analyzed the drug delivery mode and regulatory mode of TMP combined with RA in the treatment of skin photoaging. The regulation of Nrf2/ARE by HIF-1 α is a key pathway for the combination of TMP and RA to alleviate skin photoaging. This is mainly due to the high expression of HIF-1 α , which reduces the accumulation of ROS in the body and thus reduces the degree of aging of skin cells. TMP is a potential therapeutic drug for skin photoaging. Our study provides a new treatment strategy for the recovery of clinical skin photoaging.

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Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

CW conducted the experiments and wrote the paper; FJ and ZX analyzed and organized the data; WY conceived, designed the study and revised the manuscript.

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References

- Gromkowska-Kępką KJ, Puścion-Jakubik A, Markiewicz-Żukowska R, Socha K (2021) The impact of ultraviolet radiation on skin photoaging - review of in vitro studies. *J Cosmet Dermatol* 20(11):3427–3431. doi: 10.1111/jocd.14033
- Guan LL, Lim HW, Mohammad TF (2021) Sunscreens and photoaging: A review of current literature. *Am J Clin Dermatol* 22(6):819–828. doi: 10.1007/s40257-021-00632-5
- Bocheva G, Slominski RM, Janjetovic Z, Kim TK, Böhm M, Steinbrink K, et al (2022) Protective role of melatonin and its metabolites in skin aging. *Int J Mol Sci* 23(3):1238. doi: 10.3390/ijms23031238
- Ansary TM, Hossain MR, Kamiya K, Komine M, Ohtsuki M (2021) Inflammatory molecules associated with ultraviolet radiation-mediated skin aging. *Int J Mol Sci* 22(8):3974. doi: 10.3390/ijms22083974
- Papaccio F, D Arino A, Caputo S, Bellei B (2022) Focus on the contribution of oxidative stress in skin aging. *Antioxidants (Basel)* 11(6):1121. doi: 10.3390/antiox11061121
- Pei XD, Yao HL, Shen LQ, Yang Y, Lu L, Xiao JS, et al (2020) α -Cyperone inhibits the proliferation of human cervical cancer HeLa cells via ROS-mediated PI3K/Akt/mTOR signaling pathway. *Eur J Pharmacol* 883:173355. doi: 10.1016/j.ejphar.2020.173355
- Li X, Shi J, Teng Y, Liu Z (2024) The preventative effect of Baihe Gujin Pill on cisplatin-induced acute kidney injury by activating the PI3K/AKT and suppressing the NF- κ B/MAPK pathways. *J Ethnopharmacol* 318(Pt B):117071. doi: 10.1016/j.jep.2023.117071
- Lai Y, Zhang Y, Zhang H, Chen Z, Zeng L, Deng G, et al (2024) Modified Shoutai Pill inhibited ferroptosis to alleviate recurrent pregnancy loss. *J Ethnopharmacol* 319(Pt 2):117028. doi: 10.1016/j.jep.2023.117028
- Yuan F, Lu F, Guo Y, Zhang C (2023) Acupoint application combined with acupoint massage for treating constipation in a patient with chronic obstructive pulmonary disease. *J Vis Exp* (198). doi: 10.3791/64900
- Ali BH, Al-Moundhri M, Eldin MT, Nemmar A, Al-Siyabi S, Annamalai K (2008) Amelioration of cisplatin-induced nephrotoxicity in rats by tetramethylpyrazine, a major constituent of the Chinese herb *Ligusticum wallichii*. *Exp Biol Med (Maywood)* 233(7):891–896. doi: 10.3181/0711-RM-315
- Vatte S, Ugale R (2023) HIF-1, an important regulator in potential new therapeutic approaches to ischemic stroke. *Neurochem Int* 170:105605. doi: 10.1016/j.neuint.2023.105605
- Gao H, Nepovimova E, Heger Z, Valko M, Wu Q, Kuca K, Adam V (2023) Role of hypoxia in cellular senescence. *Pharmacol Res* 194:106841. doi: 10.1016/j.phrs.2023.106841
- Zhang C, Lv J, Qin X, Peng Z, Lin H (2022) Novel antioxidant peptides from *Crassostrea hongkongensis* improve photo-oxidation in UV-induced HaCaT cells. *Mar Drugs* 20(2):100. doi: 10.3390/md20020100
- Zhu S, Qin W, Liu T, Liu T, Ma H, Hu C, et al (2022) Modified Qing'e Formula protects against UV-induced skin oxidative damage *via* the activation of Nrf2/ARE defensive pathway. *Front Pharmacol* 13:976473. doi: 10.3389/fphar.2022.976473
- Jiang R, Xu J, Zhang Y, Liu J, Wang Y, Chen M, et al (2022) Ligustrazine alleviates psoriasis-like inflammation through inhibiting TRAF6/c-JUN/NF κ B signaling pathway in keratinocyte. *Biomed Pharmacother* 150:113010. doi: 10.1016/j.biopha.2022.113010
- Ju XD, Deng M, Ao YF, Yu CL, Wang JQ, Yu JK, et al (2010) The protective effect of tetramethylpyrazine on cartilage explants and chondrocytes. *J Ethnopharmacol* 132(2):414–420. doi: 10.1016/j.jep.2010.08.020
- Zhu Y, Wang K, Jia X, Fu C, Yu H, Wang Y (2024) Antioxidant peptides, the guardian of life from oxidative stress. *Med Res Rev* 44(1):275–364. doi: 10.1002/med.21986
- Kim JE, Kim WH, Kim S, Na Y, Choi J, Hong YD, et al (2023) Bioconversion of retinol and its cell barrier function in human immortalized keratinocytes cells and artificial epidermis-dermis skin. *Exp Dermatol* 32(6):822–830. doi: 10.1111/exd.14781
- Xu Z, Gong B, Li Z, Wang Y, Zhao Z, Xie L, et al (2023) Bazi Bushen alleviates skin senescence by orchestrating skin homeostasis in SAMP6 mice. *J Cell Mol Med* 27(18):2651–2660. doi: 10.1111/jcmm.17833
- Han S, Liu P, Yan Q, Cen Y, Wu G, Chen Z, et al (2024) Seawater pearl hydrolysate inhibits photoaging via decreasing oxidative stress, autophagy and apoptosis of Ultraviolet B-induced human skin keratinocytes. *J Cosmet Dermatol* 23(1):256–270. doi: 10.1111/jocd.15916
- Kim B, Kim J, Kim A, Kim YS, Lee YR, Bae YM, et al (2004) Ligusticum wallichii-induced vasorelaxation mediated by mitogen-activated protein kinase in rat aortic smooth muscle. *J Ethnopharmacol* 90(2-3):397–401. doi: 10.1016/j.jep.2003.11.003
- Lin J, Wang Q, Zhou S, Xu S, Yao K (2022) Tetramethylpyrazine: A review on its mechanisms and functions. *Biomed Pharmacother* 150:113005. doi: 10.1016/j.biopha.2022.113005
- Wu X, Wang Z, Wu G, Xu X, Zhang J, Li Y, et al (2020) Te-

- tramethylpyrazine induces apoptosis and inhibits proliferation of hypertrophic scar-derived fibroblasts *via* inhibiting the phosphorylation of AKT. *Front Pharmacol* 11:602. doi: 10.3389/fphar.2020.00602
24. Alalaiwe A, Hung CF, Leu YL, Tahara K, Chen HH, Hu KY, Fang JY (2018) The active compounds derived from *Psoralea corylifolia* for photochemotherapy against psoriasis-like lesions: The relationship between structure and percutaneous absorption. *Eur J Pharm Sci* 124:114–126. doi: 10.1016/j.ejps.2018.08.031
25. Yu P, Wei H, Li K, Zhu S, Li J, Chen C, et al (2022) The traditional Chinese medicine monomer Ailanthone improves the therapeutic efficacy of anti-PD-L1 in melanoma cells by targeting c-Jun. *J Exp Clin Cancer Res* 41(1):346. doi: 10.1186/s13046-022-02559-z
26. Zhao W, Zheng L, Yang J, Li Y, Zhang Y, Ma T, Wang Q (2023) Dissolving microneedle patches-mediated percutaneous delivery of tetramethylpyrazine for rheumatoid arthritis treatment. *Eur J Pharm Sci* 184:106409. doi: 10.1016/j.ejps.2023.106409
27. Duscher D, Maan ZN, Hu MS, Thor D (2020) A single-center blinded randomized clinical trial to evaluate the anti-aging effects of a novel HSFTM-based skin care formulation. *J Cosmet Dermatol* 19(11):2936–2945. doi: 10.1111/jocd.13356
28. Pagani A, Aitzetmüller MM, Brett EA, König V, Wenny R, Thor D, et al (2018) Skin rejuvenation through HIF-1 α modulation. *Plast Reconstr Surg* 141(4):600e–607e. doi: 10.1097/PRS.0000000000004256
29. Bonham CA, Kuehlmann B, Gurtner GC (2020) Impaired neovascularization in aging. *Adv Wound Care (New Rochelle)* 9(3):111–126. doi: 10.1089/wound.2018.0912
30. Wen W, Yang L, Wang X, Zhang H, Wu F, Xu K, et al (2023) Fucoidan promotes angiogenesis and accelerates wound healing through AKT/Nrf2/HIF-1 α signalling pathway. *Int Wound J* 20(9):3606–3618. doi: 10.1111/iwj.14239
31. Xu X, Wang S, Zhou D, Qu J, Zhang C, Xu Y, Sun L (2022) The Haoqin-Huaban formula alleviates UVB-induced skin damage through HOXA11-AS-mediated stabilization of EZH2. *Am J Transl Res* 14(4):2212–2230.
32. Yang M, Zhao M, Xia T, Chen Y, Li W, Zhang H, et al (2023) *Phytolacca alkekengi* L. var. *franchetii* combined with hormone therapy for atopic dermatitis. *Biomed Pharmacother* 162:114622. doi: 10.1016/j.biopha.2023.114622
33. Liggins MC, Li F, Zhang LJ, Dokoshi T, Gallo RL (2019) Retinoids enhance the expression of cathelicidin antimicrobial peptide during reactive dermal adipogenesis. *J Immunol* 203(6):1589–1597. doi: 10.4049/jimmunol.1900520