THE ANTI-PHOTOAGING ACTIVITY OF DEEP SEA WATER ON HACAT KERATINOCYTE INDUCED BY UVB VIA SUPPRESSION MMPS AND MAPKS/NF-κB SIGNALING PATHWAY

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Abstract: Ultraviolet radiation may cause oxidative stress, inflammation, collagen degradation, and other skin damage. In this study, the photoaging model of the human immortal keratinocyte line (HaCaT) was established by UV irradiation, and the expression of cytokines and MAPK pathway proteins was studied to evaluate the protective effect of DSW on UVB-induced cell photoaging. The results of oxidase system detection showed that DSW could significantly up-regulate the UVB-induced overexpression of superoxide dismutase (SOD)M, Catalase, and glutathione peroxidase (GSH-Px), meanwhile, the content of malondialdehyde was down-regulated. Cytokine expression detection results showed that DSW significantly reduced the expression levels of pro-inflammatory cytokines (TNF-α, IL-6). Western blot results showed that DSW significantly down-regulated UVB-induced overexpression of MMPs protein significantly reduced the phosphorylation level of MAPKs pathway proteins (SAPK/JNK, ERK1/2, p38 MAPK), and weakened the nuclear transposition expression of kappa-B induced by UVB. In conclusion, DSW significantly alleviates inflammatory response and oxidative stress damage in UVB-induced model HaCaT cells, and DSW can be used as potentially effective skin care and application material to reduce UVB-induced skin inflammation.

Keywords: Balanced deep-sea water, Ultraviolet B, Anti-photoaging, HaCaT keratinocyte

Skin aging is a complex systemic degradation process involving the skin and its supporting systems (1). Exposure to UV may cause sunburn, immunosuppression, inflammation, oxidative stress, non-melanoma and melanoma skin cancer, and premature aging of the skin, known as photoaging, depending on the amount and type of UV exposure and the skin type of the exposed individual (2-4). In the complex human organism, the external symptoms of photoaging are dry skin, fine and coarse wrinkles, loss of skin luster, dark yellow skin, poor elasticity, and impaired wound healing mechanisms (5-7). According to previous studies, oxidative damage is a contributor to aging which is associated with the body’s salubrity reduction caused by the accumulation of ROS (8). Moreover, long-term exposure to ultraviolet radiation can have cause serious negative effects on the appearance, structure, and function of the skin, eventually leading to photoaging and even skin cancer (9).

Aging is an irreversible natural process for every human organism, which can lead to a variety of diseases. Skin is highly sensitive to reactive oxygen species (ROS)-induced oxidative stress caused by light irradiation (10), and the inflammatory reaction and its side effects play an important role in the photoaging process of human skin. However, ROS levels are significantly increased under environmental...
stresses, such as UVB or heat exposure, resulting in significant impairment of cell function and structure. In addition, previous studies have shown that activation of lipid mediators including cyclooxygenase-2 (COX-2) is also associated with UV-induced ROS accumulation (11). In addition, the expression of pro-inflammatory factors in skin cells increases with exposure to ultraviolet light UV, which could lead to the overexpression of collagen degrading enzymes and matrix metalloproteinases (MMPs) (12). However, photoaging is further exacerbated by the accumulation of non-functional matrix components and degradation of abnormal matrix caused by various collagen-degrading enzymes that are activated by pro-inflammatory factors (13). Therefore, according to previous studies, the expression level of MMPs can be measured as a major marker of UVB-induced skin inflammation and photoaging (11, 14).

Deep seawater (DSW) is a kind of natural mineral water with multiple biological bioactivities (15), such as anti-tumor (16), anti-oxidation (17), anti-diabetes (18, 19) anti-hyperlipidemia (20, 21), and anti-inflammatory (22, 23). In addition, previous clinical trials and animal experiments have shown that the application and administration of DSW could help improve atopic eczema symptoms (12, 24). Moreover, cosmetics based on DSW were also launched in Chinese Taipei, Korea, Japan, and the United States (25). However, there are few products and researches related to DSW in mainland China, the effects of 800m depths DSW from south China sea on UVB-induced HaCaT cell inflammation and photoaging have not been validated elucidated (26). Therefore, we explored the effects of 800m depths deep seawater on UVB-induced inflammation and damage in HaCaT keratinocyte cell line, and further explored the molecular regulatory and cellular metabolic pathways of DSW on UVB-induced inflammation and photoaging of HaCaT cells.

EXPERIMENTAL

Materials

The testing kits for the determination of superoxide dismutase (SOD), malondialdehyde (27), glutathione peroxidase (GSH-Px), catalase (28), and the extraction of protein were supplied by Solarbio Bioengineering Co., Ltd (Beijing, China). The immunofluorescence secondary antibody (Alexa 488-conjugated AffiniPure IgG (H+L)) was supplied by Jackson Immunoresearch Co. (Pennsylvania, USA). The antifade nuclear dye containing 4,6-diamidino-2-phenylindole (DAPI) was supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA.). Nitrocellulose filter membrane was supplied by Pall Co. (New York, NY, USA). The cell assay lysis buffer (RIPA) was supplied by Solarbio Co. (Beijing, China). And all other reagents used in this research were analytical grade and supplied by Kangkede Chemical Reagent Co. (Tianjin, China).

Preparation of deep seawater

The deep seawater was collected during a marine expedition by our research team and the DSW was prepared as previous research (29). For the preparation of medium with multiple hardnesses, the Dulbecco’s modified eagle’s medium (DMEM) powder was dissolved in the DSW of original hardness (OHDW, hardness~3665, original ratio of Mg: Ca is 3:1) and the deep seawater of hardness 0 (H0), which were sterilized in sequence via a filtration process. Then, the original hardness DSW was diluted in sequence with DSW (H0) to prepare the hardness gradient medium (HH (hardness~1800), HM (hardness~1200), HL (hardness~600)) deep seawater. OHDW of 800 depth contained Mg (1270 mg/L), Ca (420 mg/L), Na (309 mg/L), and K (98 mg/L). The medium was diluted with the same volume DSW(H0) and DSW (H1800) groups for control and model groups. DMEM medium containing chemical purity MgSO4 and CaCl2 (the ratio of Mg: Ca~ 3:1) was prepared for 1200 hardness as Mg+Ca group.

Cell treatment and UVB irradiation

HaCaT keratinocyte was provided by the Sciences cell bank of Chinese Academy (Shanghai). The cells were maintained in DEAE medium (Gibco Company, USA) contained 100 µg/mL streptomycin, 100 IU/mL penicillin, and 10% fetal bovine serum (FBS) from Gibco Invitrogen Co. (San Diego, CA, USA). For the establishment of the photoaging cell model, PL-S 5W/2P UVB lamp (wavelength 311 nm, Philips, Netherlands) was used to irradiate cells layered with phosphate buffer saline (PBS). The radiation source was placed at a 10 cm distance (16.7 mJ/cm2) in a dark environment. After irradiation, cells were cultured in DMEM with FBS-free for 4 h.

Cell viability assay

The effects of different depths DSW on cell viability were determined by MTT assay. After UVB radiation, the medium with FBS-free was removed, and 200 µL condition medium was added to each well, and the cells were incubated for 24 h with DSW of different hardness (H0-H1800). Subsequently, the medium was removed, 200 µL per well methyl thiazolyl tetrazolium (MTT) solution (0.5 mg mL−1) was added, and cells were incubated for 4 hours in 5%
(v/v) CO₂ at 37°C. After MTT solution was removed, the 100 μL dimethylsulfoxide (DMSO) per well was added to melt the formazan crystals, by shaking with a 96-well plate oscillator for 15 min to maximum dissolution. The absorption of A570 was measured by a Thermo Scientific Multiskan FC (Waltham, MA, USA). The value of cell viability was performed by statistical analysis software SPSS 17.0 using the independent t-test.

**SOD, CAT, GSH-Px, and MDA determination**

5×10⁴ cells per well were plated into a 6-well plate. Cell culture and sample treatment methods refer to above 2.4. The cells were treated with protease and collected with PBS, and then the plate was centrifuged at 2500 rpm for 10 min at 4°C. The supernatants were collected to evaluate the activities of antioxidant enzymes (SOD, CAT, and GSH-Px) and the level of MDA by using the test kits.

**Immunofluorescence analysis**

The ROS production was measured by using an immunofluorescence experiment with quantitative analysis. Cells were incubated on a 6-well plate with a glass slide per plate at the density of 1×10⁵ with DSW (HL, HM, HH) and Mg²⁺Ca, then incubated for 24 h, followed by irradiation with UVB. Cell culture slides were blended with the working solution for 30 min at 37°C, then the cell slides were immersed in ROS dye for 30 min in 5% (v/v) CO₂ at 37°C. Then, the slides were washed with PBS solution twice and stained with nuclear dye for 10 min. After washing with PBS twice, the ROS production was evaluated through an immunofluorescence experiment with quantitative analysis. The slides were imaged by Laser Scanning Confocal Microscope (FLUOVIEW FV3000, Olympus Co., Tokyo, Japan).

**Total RNA extraction and purification**

The total RNA was extracted and purified with a Trizol RNA isolation assay kit from Solarbio Co. (Beijing, China). 1 mL per well of Trizol reagent was added and the mixture was reacted for 5 min. The lysates from each well were transferred to tubes and centrifuged for 15 min at 14000 rpm. After centrifuging, the supernatant was transferred to another tube to precipitate RNA. Isopropanol of equal volume was added and mixed into each tube and then the mixture was centrifuged at 13000 rpm for 10 min, after supernatant removal ethanol solution 75% (v/v) was added and centrifuged at 12000×g for 5 min. Finally, the RNA sediment was dried at room temperature and redissolved in the buffer solution treated with 50 μL diethyl pyrocarbonate (DEPC).

**Expression detection of mRNA gene using qRT-PCR**

The qRT-PCR analysis was performed to evaluate the expression level of IL-6 and TNF-α. According to the manufacturer’s protocol of AceQTM qPCR SYBR Green Master Mix (Agilent Corp., CA, USA), the reaction solution was prepared with 1 μL RNA template, 0.1 μL forward/reverse primer, 10 μL 2×SYBR qPCR mix, 2 μL of 50×ROX reference dye, and deionized distilled H₂O was added to adjust the total volume to 20 μL/well. The ABI StepOnePlus (Thermo Fisher Scientific, MA, USA) was set up as follows, firstly the initial temperature was set up at 95°C for 15 min for one cycle. Then the temperature was set up at 95°C for 10 s, annealing at 60°C for 20 s, extension at 72°C for 30 s, and the above program was set for 41 cycles. The designed primers of forward and reverse from 5′ to 3’ were shown in S-1.

**Western blot analysis**

Cells were collected in cell lysis buffer (RIPA) containing protease inhibitor and phosphatase inhibitor, kept on ice for 30 min. Cell lysates were collected and centrifuged at 15000 rpm for 15 min at 4°C. After that, the supernatants were transferred to another tube as total protein. The total protein solution was measured via the BCA protein quantitation kit. An equal number of proteins was separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to the nitrocellulose (NC) membranes. After that, the membranes were blocked in tris-buffered saline (TBST) containing 5% skim milk powder. Then, the membrane was incubated with primary antibodies diluted with TBS containing 1% BSA solution overnight at 4°C, after reaction with primary antibodies membrane was washed with TBST three times for 5 min each time. After that, the NC membrane was incubated with secondary antibodies conjugated horseradish peroxidase (HRP) for 1h. After washing the membrane with TBST three times, the chemiluminescent reaction substrate for HRP was dropped onto NC membranes. Finally, the Bioimaging Systems iBright CL750 (Thermo Fisher, MA, USA) was used to image and photograph the protein band. The fluorescence intensity of protein bands was analyzed by using Thermo Fisher ABI qubit4 (Thermo Fisher, MA, USA).

The primary antibodies are described as follows: MMP-1 (E9S9N) Rabbit mAb, MMP-2 (D8N9Y) Rabbit mAb, MMP-9 (D6O3H) XP® Rabbit mAb, COX-2 (D5H5) XP® Rabbit mAb, Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb,
SAPK/JNK Antibody, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb, p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb, Phospho-P38 MAPK (D3F9) XP® Rabbit mAb, P38 MAPK (12F8) Rabbit mAb, NF-κB p65 (D14E12) XP® Rabbit mAb #8242, GAPDH (D16H11) XP® Rabbit mAb. The secondary antibodies are described as follows: HRP-conjugated/fluorescent IgG for secondary antibodies. The GAPDH was used as the internal standard.

Statistical analyses
All values were expressed as mean ± standard deviations (mean ± SD) and at least three times. Statistical analysis was performed by using the independent t-test in the statistical analysis of SPSS 25.0 statistical analysis software. And p-value < 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Effect of DSW from multiple depths on cell viability of HaCaT cells induced by UVB

In this study, the effects of DSW from multiple depths on cell viability in UVB-induced HaCaT cell line. As Figure 1 showed, it can be confirmed that DSW of 200 m and 800 m in 600, 1200, and 1800 hardness ranges enhanced cell viability according to the slight inhibition of cell proliferation induced by UVB. In contrast, DSW improved UVB-induced cell viability in a hardness-dependent manner, which can be seen from the cell viability of UVB-induced HaCaT. Furthermore, as Figure 2 showed, the protective effect of DSW on cell viability might be associated with the decrease of ROS expression in damaged HaCaT cells, which might also be related to the suppression effect of DSW on UVB-induced inflammation pathways.

Effect of DSW on levels of SOD, CAT, GSH-Px, MDA, and expression of ROS in UVB-induced HaCaT cells

The generation of ROS induced by UVB plays an important role in the process of skin photoaging. As Figure 2 showed the immunofluorescence method was used to evaluate the ROS generation. What can be seen from Figure 2, the ROS expression of the model control group was significantly higher than the normal control group. However, the hardness of 600, 1200, and 1800 groups showed a significant reduction of ROS expression. Among them, the inhibition effect was most obvious in HM group of 1200 hardness. It indicates that the DSW of proper hardness could inhibit the generation of ROS to protect HaCaT cells from oxidative stress damage, which also showed a dose-dependence between DSW activity and hardness.

Excessive ROS generation is related to ultraviolet irradiation, which caused oxidative stress and inflammatory response. The antioxidant enzymes such as SOD, CAT, and GSH-Px can remove the excess of accumulated ROS and repair cellular state from oxidative and immune stress. Compared with the normal control group, the antioxidant enzymes (SOD, CAT, and GSH-Px) in the model group were significantly decreased by 30%, 50%, and 11%, respectively (Table 1). The UVB-induced decrease of antioxidant enzymes (SOD, CAT, and GSH-Px) activities was increased significantly by DSW of various hardness and depth compared with the model group. However, compared with D200 (HL, HM, and HH) group, antioxidant enzymes of D800 (HL, HM, and HH) group showed no significant difference.

Excessive ROS can lead to lipid peroxidation, which leads to oxidative damage of cells. MDA is the by-product of lipid peroxidation, which is also induced by ROS. As Table 1 showed, compared with the normal group, the MDA level of the model group was significantly increased by nearly 30%. Compared with the model group, the MDA levels of UVB-DSW treatment groups were significantly inhibited, respectively ($P < 0.05$). However, no statistically significant
Figure 2. Effect of DSW with multiple harnesses on the expression of ROS of UVB-induced HaCaT keratinocyte. Cells were inoculated into 6 well-culture dishes. Cells were incubated at 37°C for 24 h and then were exposed with UVB at a 10-cm distance, after that, cells were treated with DMEM medium supplemented with model and various hardness levels of DSW with FBS-free; then the cells were further incubated in 37°C for 24 h. ROS expression induced by UVB was decreased by treatment with various hardness levels of DSW of 800m depth. Cells were fixed with 4% paraformaldehyde-PBS solution, stained by antifade nuclear dye (DAPI), and observed and imaged through a fluorescent microscope. The up-regulated expression of ROS induced by UVB in HaCaT keratinocytes was inhibited by DSW treatment (green). 500 × Magnification.

Table 1. Effect of DSW on the expression of oxidation-related enzymes in HaCaT cell line

| Group   | Hardness | SOD (U/mg prot) | CAT (U/mg prot) | GSH-Px (U/mg prot) | MDA (U/mg prot) |
|---------|----------|-----------------|-----------------|-------------------|-----------------|
| Normal  | 0        | 47.85 ± 2.98    | 2.23 ± 0.17     | 49.35 ± 3.27      | 3.42 ± 0.08     |
| Model   | 0        | 32.13 ± 2.93#   | 1.11 ± 0.15#    | 42.15 ± 1.95#     | 5.01 ± 0.13#    |
| HL D200 | 600      | 42.11 ± 0.59*   | 1.39 ± 0.25*    | 43.67 ± 1.83*     | 4.46 ± 0.22*    |
| HL D800 | 600      | 43.26 ± 1.43*,b | 1.45 ± 0.16*,b  | 44.50 ± 0.50*,b   | 4.37 ± 0.25*,b  |
| HM D200 | 1200     | 44.10 ± 2.12**,b| 1.88 ± 0.18**,b | 48.54 ± 1.58**    | 3.94 ± 0.12**   |
| HM D800 | 1200     | 45.95 ± 4.01**,b| 1.92 ± 0.15**,b | 49.05 ± 1.99**,b  | 3.71 ± 0.04**,b |
| HH D200 | 1800     | 41.12 ± 1.75*   | 1.32 ± 0.16*    | 42.25 ± 1.25*     | 4.41 ± 0.10*    |
| HH D800 | 1800     | 42.32 ± 1.75*,b | 1.37 ± 0.14*,b  | 43.17 ± 1.35*,b   | 4.36 ± 0.15*,b  |
| Mg+Ca   | 1200     | 43.75 ± 1.45**,b| 1.78 ± 0.12**,b | 47.98 ± 0.72**,b  | 4.08 ± 0.17**,b |

Data were presented as mean ± SD (n=6). Data with different letters in the same column implied significant difference. *P < 0.05, **P < 0.01 and ***P < 0.005 compared to normal control group only. *P < 0.05, **P < 0.01 and ***P < 0.005 compared to model control group only, b no significant, group D800 compared with group D200 of the same hardness.
difference was discovered between the depth of 200 m and 800 m in the same hardness group.

The results showed that DSW effectively reduced oxidative stress of the HaCaT keratinocyte by inhibiting lipid peroxidation. Moreover, the activity of DSW was significantly correlated with its hardness, while the activity was not significantly correlated with its depth.

Effect of DSW on MMPs levels in UVB-induced HaCaT cells

Photoaged skin has been shown to contain high levels of matrix metalloproteinases, which were considered to be a sign of photaging due to their collagen-digesting function (30-33). As seen in Figure 3 the expression levels of MMP-1 in the model control group increased 3.21-fold higher than the normal

![Graphs showing MMP-1, MMP-2, MMP-9, and COX-2 expression levels with DSW treatment](image)

Figure 3. A) Effect of DSW on MMPs and COX-2 expression in UVB-induced HaCaT keratinocyte was examined by western blotting. B) The protein expressions levels of MMP-1(A), MMP-2(B), MMP-9(C), and COX-2(D) were quantified using Image Lab statistical software. Cells were incubated at 37°C for 24 h and then were exposed with UVB at a 10-cm distance, after that, cells were treated with DMEM medium supplemented with model and various hardness levels of DSW with FBS-free; then the cells were further incubated in 37°C for 24 h. Data were expressed as the mean ± SD of six independent experiments. (*P < 0.05, **P < 0.01 and ***P < 0.005 compared to model control group only, ##P < 0.01 MC compared with NC, a P < 0.01 HM compared with Mg+Ca, b P no significant, HM compared with Mg+Ca).
control group. However, the expression level of MMP-1 in the DSW group was significantly decreased by 46.5% compared with the model control group. Although the expression levels of MMP-1 in DSW-1800 groups were not significantly different compared with the model control group, which also reduced by 13.79%. The results in Figure 3 shows that DSW effectively improved the overexpression of metalloproteinase (MMPs-1 and MMPs-2). Densitometric analysis of western blotting bands showed, compared with non-UVB exposed cells, UVB exposure induced up-regulation of MMP-1, MMP-2, and MMP-9 expression as the results shown in Figure 3. In addition, previous studies have shown that the COX-2 is a lipid mediator activated by UV irradiation which is an important mediator of photoaging (34, 35). Here, the results in Figure 3 indicated that DSW had significant effects on inhibition of UVB-induced COX-2 overexpression. In summary, all the results indicated that DSW down-regulated UVB-induced overexpression of COX-2, MMP-1, MMP-2, or MMP-9, which provided further support to the potential anti-photoaging activity of DSW.

Effect of DSW on TNF-α and IL-6 expressions in UVB-induced HaCaT cells

The effect of DSW on UVB-induced the mRNA expression of pro-inflammatory cytokines (IL-6 and TNF-α) in HaCaT induced by UVB were determined by qPCR. The results shown in Figure 4 revealed that the mRNA expression of pro-inflammatory cytokines (IL-6 and TNF-α) were significantly enhanced in the model control group compared with the normal control group ($P < 0.05$). However, compared with the model control group, the mRNA expression levels of IL-6 and TNF-α were significantly decreased by incubation with DSW, which also showed dose-dependency. It indicated that DSW might protect the HaCaT cell line from photoaging by reducing the inflammatory response.

Effect of DSW on the expression of MAPK signal pathway protein in UVB-induced HaCaT cells

MAPKs have been known as major to synthesis and release of pro-inflammatory cytokines and mediators in the inflammatory process, which might be relative to the photoaging of UVB-induced HaCaT cells. To determine whether the anti-inflammatory and anti-photoaging activities of DSW are mediated by the MAPK pathway, the phosphorylation expression of JNK (p-JNK), ERK (p-ERK), and p38 MAPK (p-p38) were examined by western blot analysis. HaCaT cells were treated with different hardness and manual preparation of calcium and magnesium solution. Figure 5 showed that the expression of activation phosphorylation MAPKs (p-JNK, p-ERK, p-p38) that occurred in UVB-induced HaCaT cells was low in the control group. Treatment of UVB-induced phosphorylation of p38, ERK 1/2, and JNK and treatment of DSW inhibited phosphorylation of p38, ERK 1/2, and JNK in a hardness-dependent manner, which also occurred in the Mg+Ca group. Furthermore, no significant changes were observed in expressions of total proteins of MAPKs in DSW groups. These results indicated that appropriate proportions of Mg$^{2+}$/Ca$^{2+}$ solution can regulate the phosphorylation expression of MAPKs protein to alleviate the inflammatory response in UVB-induced HaCaT cells. It is worth mentioning that the expression level of phosphorylated MAPKs in the HMDSW group was significantly lower than that in the Mg+Ca

![Figure 4](image1.png)  
![Figure 5](image2.png)

Figure 4. Effect of DSW on IL-6 and TNF-α mRNA expression in UVB-induced HaCaT keratinocyte was examined by Q-PCR. Cells were incubated at 37°C for 24 h and then were exposed with UVB at a 10-cm distance, after that, cells were treated with DMEM medium supplemented with model and various hardness levels of DSW with FBS-free; then the cells were further incubated in 37°C for 24 h. Data were expressed as the mean ± SD of six independent experiments. (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$ compared to model control group only, ##$P < 0.01$ MC compared with NC, *$P < 0.01$ HM compared with Mg+Ca, /$P$ no significant, HM compared with Mg+Ca).
group, which indicated that the deep seawater has better anti-inflammatory activity than Mg/Ca solution with the same hardness and could improve the photoaging induced by UVB. This may be due to the abundance of minerals, trace elements, and small peptides in DSW.

**Effect of DSW on NF-κB in UVB-induced HaCaT cells**

NF-κB is generally inactive in the cytoplasm, which is also a downstream target of the MAPK signal pathway (36). Upon receipt of NF-κB activation signals, the serine residue in IκB was separated from NF-κB and transferred into the nucleus as an activated transcription factor. After degradation of IκB, the binding site of the p50-P65 dimer was exposed to bind to the IκB motif. Then the NF-κB p65 subunit transfers from the cytoplasm to the nucleus and in turn appear to be potent activity.

As shown in Figure 6, compared with the normal control group, the protein in the nucleus of NF-κB p65 in the model control group was significantly enhanced with UVB irradiation. The DSW of hardness 1800 (HL) and hardness 1200 (HM) significantly inhibited the nuclear translocation of NF-κB expression by 34.51% and 55.26% in UVB-induced HaCaT cells, respectively. Meanwhile, appropriate proportions of Mg$^{2+}$/Ca$^{2+}$ solution can down-regulate the expression of nucleus NF-κB p65 in UVB-induced HaCaT cells. Compared with the group of Mg$^{2+}$/Ca$^{2+}$, DSW with hardness 1200 (HM) showed significant inhibitory activity on the expression of nucleus NF-κB p65. In summary, the DSW might prevent skin from inflammation invading through its inhibitory effect on the translocation of NF-κB from the cytoplasm to the nucleus.

**DISCUSSION**

As the body’s largest organ, skin is always subjected to multiple external conditions. Therefore, its defense against these harmful stresses is crucial to
UVB is one of the external sources of skin damage. Previous studies (37) have shown that skin inflammation caused by prolonged UVB exposure is associated with the development of skin photoaging. The purpose of this study was to investigate the preventive effect of DSW on UVB-induced photoaging and to explore the probable mechanisms by the HaCaT cell model induced by UVB.

Oxidative stress can be caused by both internal resources and the external environment, some of the primary damage is caused by peroxides. A lack of antioxidants can lead to oxidative stress and excessive accumulation of free radicals, mutations in peroxide membranes, and even deoxyribonucleic acid, and lead to structural changes and the functional abnormalities of proteins. ROS attacks and reacts with the stable molecular structure of skin cells, leading to cross-linking of elastin and collagen, while affecting the skin's self-repairing capability (38). In this study, as Figure 1 and Figure 2 showed, we studied the antioxidant effects and ability to reduce ROS production induced by UVB. The beneficial effect of DSW might be related to its inhibition of the excessive production of ROS under UVB radiation.

Generally, the human body has antioxidant enzymes including SOD, GSH-Px, and CAT, which are the body's first antioxidant defense system to prevent the accumulation of free radicals and repair oxidative damage. Previous studies and research have shown that ROS seriously damage the skin's antioxidant defense system, and the mechanism of UVB-induced photodamage is related to the excessive generation of ROS, which leads to accompanying oxidative stress. Lipid peroxidation is one of the main causes of oxidative stress, which also significantly increased in HaCaT cells induced by UVB. MDA is a degradation product of lipid peroxidation which could be induced by UVB and be widely regarded as one of the important biomarkers of oxidative stress. In this study, as Table 1 showed, DSW could significantly enhance the antioxidant enzymes activities of SOD, GSH-Px, CAT induced by UVB and inhibit the up-regulation of MDA level.

Collagen is the main structural component of the dermal extracellular matrix, and the degradation and destruction of collagen is the fundamental cause of skin photodamage. Collagen degradation is the cause of skin wrinkles and sagging, which is...
caused by overexpression of matrix metalloproteinases (MMPs). In addition, due to the decreased skin moisture content induced by UVB-radiation, which also caused the dullness and roughness, the irregular thickening of skin epidermis, resulted in wrinkles and roughness of the skin (39). In this study, DSW was demonstrated to significantly inhibit the expression of MMP-1, MMP-2, and MMP-9 proteins (Figure 3). These results indicated that DSW has potential protective effect against UVB-induced skin cell photoaging.

Mitogen-activated protein kinases (MAPKs), including JNK, ERK, and p38, are generally defined to be important regulators of cellular pathway signaling from extracellular to nucleus and play an important role in the process of cellular differentiation, proliferation, and apoptosis. The oxidative stress induced by UVB plays an important role in the activation of the MAPK pathway. According to previous reports, the oxidative stress induced by UVB-irradiation mediates the phosphorylation levels of MAPKs including JNK, ERK, and p38, which are associated with photoaging (40). However, no studies have confirmed that DSW could play an anti-aging role in the inhibition of the MAPK signaling pathway. Therefore, in this study, the results demonstrated that DSW might alleviate UVB-induced oxidative stress which led to the inhibition of the MAPK signaling pathway, which plays a critical role of DSW in the prevention and treatment of photoaging.

Previous studies have reported that the activation of phosphorylation MAPK proteins could mediate the downstream NF-κB. NF-κB is a transcription factor that can be activated by ROS induced by UVB irradiation which plays a critical role in all stages of carcinogenesis. It has been reported that the activation of MAPK and NF-κB could promote and accelerate the expression of MMPs. In this study, the expression MMPs induced by UVB was inhibited with DSW treatment by inhibiting the phosphorylation of MAPK proteins and translocation of NF-κB. In this study, DSW was demonstrated to reduce the translocation of NF-κB and phosphorylations of MAPKs in the HaCaT keratinocyte with UVB-exposed (Figure 5). Furthermore, the expression of cytosol-NF-κB was down-regulated (Figure 6). Therefore, the results demonstrate that the DSW mitigated the decrease of cell viability exposed to UVB-irradiated by inhibiting the excessive accumulation of ROS and the overexpression of MMPs through the NF-κB and MAPKs signaling pathways, thereby inhibiting oxidative stress, inflammatory response, and collagen matrix degradation. Activation of NF-κB could upregulate the expressions of TNF-α and IL-6, which are important regulators of inflammation response. In this study, as Figure 4 showed, DSW with appropriate hardness could effectively inhibit the mRNA expression of IL-6 and TNF-α, and inhibit the activation and translocation of NF-κB in UVB-induced HaCaT keratinocyte.
Here, we investigated the protective effects of 200m and 800m DSW depth of 600, 1200, 1800 hardness, and Mg+Ca on UVB-induced HaCaT keratinocyte against photoaging. The results showed that decreased cell viability induced by UVB was tied by DSW and Mg+Ca. However, there was no significant difference in the activity of seawater at different depths of 200 and 800 m. DSW contains an abundant amount of Mg. Ca is another major component found in DSW, and the ratio of calcium and magnesium ions is 3:1. In order to study the anti-inflammatory and anti-photoaging effect of Mg, Ca and other trace elements in DSW, group Mg+Ca with the same hardness contained the same proportion (3:1) of magnesium and calcium as the reference. As Figure 1 showed, the DSW significantly improved the cell survival rate reduction induced by UVB, and the 1200 hardness DSW of 200 m and 800 m significantly improved the cell survival rate reduction, which was significantly better than the Mg+Ca group. However, there is no significant difference between the activity of seawater at a depth of 200 m and 800 m, this may be due to the unique properties of deep water, such as cleanliness, do not play a dominant role in its display of anti-photoaging activity. As Figure 2 and Table 1 showed, both the group of DSW HM and Mg+Ca could obviously improve the expression of oxidation-related enzymes and downregulation the expression of ROS. As Figure 3 showed, DSW and Mg+Ca all can up-regulation/down-regulation the expression of MMPs and COX-2, DSW had significantly better regulatory effects on expression of MMP-1, MMP-9, COX-2 protein than Mg and Ca solutions. As shown in Figure 4 and Figure 5A, Mg+Ca also down-regulation the MAPKs expression and nucleus-NF-κB in UVB-radiated HaCaT keratinocyte. Therefore, the result suggests the major role of Mg and Ca on the suppression of inflammatory response in UVB-radiated HaCaT keratinocytes. However, under the same concentration of magnesium and calcium ions, the anti-inflammatory and anti-photoaging activity of DSW is significantly better than Mg+Ca solution. This may be due to the DSW being rich in a variety of trace element ions.

Collectively, as Figure 7 showed, the protective mechanism of DSW against photoaging may be that it mediates the expression of cytokines and MMPs in UVB-irradiated HaCaT keratinocytes by up-regulating/down-regulating the protein expression of MAPK and NF-κB signaling pathway. The conclusion suggests that DSW can be potentially used for skin health care through the remission of photoaging aging skin cells.

CONCLUSIONS

Our study demonstrated that the DSW from the South China Sea could ameliorate the inflammation, collagen degradation, and aging induced by UVB. The mechanism may be the inhibition of UVB-induced oxidative stress, inflammatory response, and MMP-1 expression by regulating MAPK and NF-κB signaling pathways. Therefore, the DSW might be a potential cosmetic or medical material for skin health against anti-photoaging.

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Conflict of interest

The authors declare no conflict of interest. REFERENCES

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