Protective effects of κ-ca3000 + CP against ultraviolet-induced damage in HaCaT and MEF cells

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ARTICLE INFO

Article history:
Received 27 April 2010
Accepted 21 June 2010
Available online xxxx

Keywords:
Peptide–oligosaccharide complex
Antioxidant
Photoprotection
UV-irradiation

ABSTRACT

In this study, the complex κ-ca3000 + CP combined collagen peptide with κ-carrageenan oligosaccharide was tested for its ability to moderate UV-induced damage and investigated for its protective mechanism against UV radiation. Human keratinocytes (HaCaT) and mouse embryonic fibroblasts (MEF) were used to monitor the effects of the κ-ca3000 + CP on cell viability, apoptosis, level of collagen I and MMP-1, MAPKs activation and intracellular ROS production after UV-irradiation. The results indicated that application of the κ-ca3000 + CP (100 μg/ml) could significantly attenuate UV-induced HaCaT and MEF death, as well as inhibit the UV-induced apoptosis of HaCaT cells. The decreased collagen I synthesis and the increased MMP-1 expression of MEF by UV radiation were almost restored back to normal level after treatment with κ-ca3000 + CP. Moreover, κ-ca3000 + CP could significantly suppress UV-induced MAPKs activation and intracellular ROS production. Taken together, these results showed that antioxidant property of κ-ca3000 + CP can effectively attenuate UV-caused cell damage and skin photoaging by suppressing cell apoptosis and expression of MMP-1 through the MAPKs signaling pathways. Thus, κ-ca3000 + CP has potential antiaging effects and prominent protective effects on UV-induced skin cell damages, which might be used in pharmaceutical and cosmetic industries.

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1. Introduction

High dose of UV-irradiation is often known to be one of the most ubiquitous environmental hazards that impact every living creature under the sun. Skin is the largest human organ, and is the only organ directly exposed to UV-irradiation [1,2]. A growing body of evidence suggests that reactive oxygen species (ROS) are generated by UV radiation, especially for UVB (290–320 nm) and UVA (315–400 nm) radiation, resulting in oxidative damage to both epidermal and dermal cells [2–4]. Severe oxidative stress may result in photoaging, and even apoptotic or necrotic skin cell death [3–5]. Thus, application of non-toxic and high-performance antioxidants could be a successful strategy for protecting the skin against ROS-induced injury [6].

Oceans are important natural resources for several peptides and oligosaccharides with potential antioxidant properties. In our previous study, κ-carrageenan oligosaccharides with high contents of sulphate groups showed strong antioxidant activities, especially for the oligosaccharides with molecular weight of about 3 kDa (κ-ca3000). Hou et al. [7] reported the bioactive tilapia skin collagen peptides (CP) rich in hydroxyl and carboxyl groups exhibited antioxidant activities and protective functions against ultraviolet radiation (UV) induced skin ailments. The specific properties of each peptide and oligosaccharide from marine active extracts offer possibilities to produce complexes that can confer unique novel properties [8]. Thus, the scavenging free radical ability and the protective effects of the peptide–oligosaccharide complex κ-ca3000 + CP against UV radiation have caused our concerns and interests.

In this study, we prepared the collagen peptide–κ-carrageenan oligosaccharide complex κ-ca3000 + CP and tested its ability to moderate photodamage, with the investigation of its protective mechanism against UV-irradiation.

2. Materials and methods

2.1. Reagents

3-(4,5-Dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma–Aldrich (USA). Dulbecco’s Modified Eagle Medium (DMEM), Modified Eagle’s Medium (MEM), penicillin, streptomycin, and 0.25% trypsin were purchased from Gibco (USA). All other reagents used were of analytical grade.

κ-Carrageenan was kindly provided by School of Medicine and Pharmacy, Ocean University of China. Bioactive tilapia skin collagen peptides (CP) rich in hydroxyl and carboxyl groups exhibited antioxidant activities and protective functions against ultraviolet radiation (UV) induced skin ailments. The specific properties of each peptide and oligosaccharide from marine active extracts offer possibilities to produce complexes that can confer unique novel properties [8]. Thus, the scavenging free radical ability and the protective effects of the peptide–oligosaccharide complex κ-ca3000 + CP against UV radiation have caused our concerns and interests.

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collagen peptide was generously provided by Chinese Center of Marine Biotechnology/BAC/UNESCO.

2.2. Preparation of peptide–oligosaccharide complex

2.2.1. Isolation and purification of the κ-carrageenan oligosaccharides

The fraction of κ-carrageenan oligosaccharides (degree of polymerization (DP) = 7–19), namely κ-ca3000, was prepared by acid hydrolysis of the 1% (w/v) corresponding block solution in 0.1 M H2SO4 at 60 °C for 1 h, and was neutralized by BaCO3 [9]. The obtained oligosaccharides were then size-fractionated by preparative gel filtration chromatography on a 100 cm × 1.5 cm Bio-Gel P6 column followed by elution with 0.1 M NH4HCO3 at a flow rate of 15 ml/h [10]. The target oligosaccharides were collected in terms of degree of polymerization (DP = 7–19) and immediately freeze-dried.

2.2.2. Determination of molecular weight

The average molecular weight of the collected fraction was measured by high performance liquid chromatography (HPLC) according to Yamamoto et al. [11] with TSK-G2000 SW column (7.5 mm × 300 mm, Japan) on Agilent 1100 LC equipped with refractive index detector (RID). The system was maintained at 35 °C and the mobile phase was 0.1 M Na2SO4 at a flow rate of 0.1 ml/min. The collected fraction was dissolved in 0.1 M Na2SO4 to a final concentration of 10 mg/ml and was filtered through 0.22 μm filter membrane before analysis. The column calibration was performed with various dextran standards (MW: 180, 1200, 2500, 4600, 7100, 10,000, and 11,700 Da) purchased from Fluka. Molecular weight of the collected fraction was calculated using the Angilent GPC software (USA).

2.2.3. Cross-linking

The standardized oligosaccharide fraction (κ-ca3000) and the bioactive tilapia skin collagen peptide (CP) were separately dissolved in double-distilled water at a concentration of 4% (w/v) and heated at 50 °C for 30 min while continuously stirring. Subsequently, the κ-ca3000 and CP were mixed in the proportion of 1:1 in order to obtain the peptide–oligosaccharide complex, namely κ-ca3000 + CP. The blended solution was stirred for 1 h at 50 °C for homogeneity and then freeze-dried [12,13].

2.3. Cell culture

Mouse embryonic fibroblast (MEF), from Kunming white foetus, was isolated according to the method of Xue[14], and cultured in MEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Generation 2–5 of MEF cells were selected for further assays.

Human keratinocyte cell line (HaCaT), purchased from China Center for Type Culture Collection (CCTCC), was cultured in MEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, and maintained at 37 °C with 5% CO2 in a humidified atmosphere.

2.4. UV-irradiation and treatment

The cells were randomly divided into different groups including control group (normal cultured cells), model group (UV-irradiated cells), compound group (different compound pretreated and UV-irradiated cells). When grown to 80–90% confluence, cells were subjected to irradiation with both UVA lamps (320–400 nm) and UVB lamps (290–320 nm) (Beijing Normal University, China) at a dose of 5 J/cm² and 15 mJ/cm² [15], which were monitored by UVA and UVB radiometers (Beijing Normal University, China) respectively.

Each compound was separately added to the cell culture medium 1 h before irradiation at different final concentrations (10, 20, 50, and 100 μg/ml). Then the mixtures were aspirated and the cells were covered with PBS during irradiation. After irradiation, PBS was replaced with the original medium and the cells were incubated for different periods at 37 °C in 5% CO2 [16].

2.5. Cell viability assays

Cell viability was evaluated by the MTT colorimetric assay as described by Alesiani et al. [17]. MTT was dissolved in PBS at 5 mg/ml. Cells were seeded in 96-well microplate (5 × 10³ cells/ml) 24 h, and then were treated according to the method described in Section 2.4. After incubation for 18 h, the cells were treated with MTT for 4 h at 37 °C in 5% CO2. After that, the medium with MTT was removed and 100 μl of dimethylsulfoxide (DMSO) was added to cells. The absorbance was measured at 490 nm in an automatic microplate photometer.

In addition, we also evaluated the effects of κ-ca3000 + CP treatment after UV-irradiation on cell viability. Prior to UV-irradiation, cells were seeded in 96-well microplate (5 × 10³ cells/ml) 24 h, and then washed and covered with PBS. After UV-irradiation PBS was removed, serum free medium with each compound (10, 20, 50 and 100 μg/ml) was applied and the cells were incubated thereafter at 37 °C for 18 h. After that, the cell viability was measured by MTT method as described above.

2.6. Modulation of UV-induced apoptosis by κ-ca3000 + CP

2.6.1. Flow cytometric analysis of cell cycle

Apoptotic cells show a diminished staining below the G0/G1 population of normal diploid cells. The DNA specific fluorochrome Propidium Iodide (PI) can identify a distinct hypo-diploid cell population, which was measured according to the method of Vermes et al. [18]. After UV-irradiation and then incubation for 18 h [16], 10 × 10⁶ HaCaT cells were trypsinized and washed with PBS by centrifuging (200g). After that, cellular DNA was stained with the PI-staining solution (PI 50 pg/ml in 0.1% sodium citrate plus 0.1% Triton X-100, Sigma) in polypropylene tubes for 15 min at room temperature in darkness. The distribution of cells in the different cell cycle phases was analyzed from the DNA histogram using a Bectone Dickinson FACS Vantage flow cytometer and Cell Quest software samples [19].

2.6.2. TUNEL assay

DNA-fragmentation in HaCaT cells was evaluated by TdT-mediated dUTP nick end labeling (TUNEL) assay [20]. After UV-irradiation and then incubation for 2 h [21], the apoptosis of cell samples was measured by TUNEL staining according to the manufacturer’s protocol of DeadEnd™ Fluorometric TUNEL assay (Promega, USA), and combined with PI-staining for cell nuclei. The green fluorescence of Fluorescein-12-UTP and the red fluorescence of PI were measured at 520 ± 20 nm and at >620 nm respectively by Laser Scanning Confocal Microscope (Zeiss LSM 510, GER).

2.6.3. Cleavage of PARP

After reaching confluence, the HaCaT cells were trypsinized and then plated on a 6-well culture dish at 5 × 10⁵ cells per well in 1 ml of MEM containing 10% FCS. After 24 h, the medium was replaced with 1 ml of serum-free MEM containing the maximum efficient concentration of κ-ca3000 + CP, which was prescreened by the cell viability assays as described in Section 2.3. Following UV-irradiation, cell samples were incubated for 12 h [22], and then lysed in cell lysis buffer (62.5 mM Tris–HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.1% w/v bromophenol blue). Equal amounts of the protein samples were subjected to 10% SDS–PAGE and the
separated proteins were then electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes. The membrane was stained with Ponceau S for control of transfer and then saturated with 5% nonfat dried milk in Tris-buffered saline (TBS) for 2 h at room temperature [23]. The blots were separately incubated overnight at 4°C with PARP and β-actin antibodies (Santa Cruz, CA, USA). After washing three times with TBST, the membrane was incubated with HRP-labeled secondary antibody and the bands were eventually visualized with chemiluminescence (ECL) kit (GE Healthcare).

2.7. ELISA assays of MMP-1 and collagen I

After reaching confluence, the MEF cells were trypsinized and then plated on a 6-well culture dish at 5 x 10^5 cells per well in 1 ml of MEM containing 10% FCS. After 24 h, the medium was replaced with 1 ml of serum-free MEM containing the maximum concentration of g-ca3000 + CP, k-ca3000 and CP, which were prescreened by the cell viability assays as described in Section 2.5. After UV-irradiation and then incubation for 18 h [16], the supernatants were collected and subjected to ELISA for MMP-1 and collagen I [24,25]. The ELISA assays were performed according to the manufacturer’s protocol of MMP-1 and collagen I assay kit (R&D, USA).

2.8. Western blot analysis of phosphorylation of JNK, ERK and p38

The treatment of the HaCaT cells and immunoblotting were the same as described in Section 2.7, with the exception that cell samples were harvested at 2 h after UV-irradiation [23]. PARP and β-actin antibodies were replaced by phospho-ERK, phospho-JNK, phospho-p38, total ERK, total JNK and total p38 antibodies (Santa Cruz, CA, USA).

2.9. Intracellular ROS measurement

The treatment of the HaCaT cells was the same as described in Section 2.7. The oxidative burst activities of cell samples were measured according to the manufacturer’s protocol of Reactive Oxygen Species Assay Kit (Beyotime Institute of Biotechnology, China). After UV-irradiation and then incubation for 2 h [26], the cells were harvested and washed twice, and then resuspended in 10 μM 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) and incubated at 37°C for 30 min. The levels of intracellular ROS were analyzed by using a Becton Dickinson FACS Vantage flow cytometer.

Fig. 1. The effects of CP, k-ca3000 and k-ca3000 + CP on cell viability in HaCaT and MEF cells. HaCaT (A and C) and MEF (B and D) cells were respectively treated with CP, k-ca3000 and k-ca3000 + CP at different final concentrations (10, 20, 50, and 100 μg/ml) before (A and B) or after (C and D) UVA (5 J/cm²) and UVB (15 mJ/cm²) irradiation. After incubation for 18 h, percent cell viability was assessed by the MTT assay. Data were expressed as the percent of non-UV-irradiated control. Values are means ± S.D. (n = 8). Significance: *p < 0.05, **p < 0.01 vs. UV model group; *p < 0.05, **p < 0.01 vs. treatment of CP or k-ca3000 group.
eter. The wavelength of excitation is 488 nm, and the fluorescence was measured at 530 nm. The level of intracellular ROS was expressed as the mean fluorescence intensity. Data acquisition and analysis were performed using Cell Quest Software [27].

3. Result

3.1. Molecular mass of oligosaccharides

The molecular mass of the collected fraction of κ-carrageenan oligosaccharides was determined by HPLC based on the calibration with dextran standards. The molecular mass of the fraction of κ-carrageenan collected in terms of DP = 7–19 (κ-ca3000) was 2926.45.

3.2. Effect of κ-ca3000, CP, and κ-ca3000 + CP on cell viability in UV-irradiated cells

The effects of κ-ca3000, CP, and κ-ca3000 + CP on the viability of UV-irradiated skin cells were assessed. HaCaT and MEF cells were pretreated with the three compounds at different final concentrations (10, 20, 50, and 100 μg/ml) for 1 h respectively. Cell viability was evaluated by the MTT colorimetric assay as described in materials and methods. As shown in Fig. 1A and B, UV-irradiation reduced HaCaT and MEF cell viabilities to approximately 60% of the control values. The survival rates of the both two cells pretreated with κ-ca3000, CP and κ-ca3000 + CP increased in a dose-dependent manner, and reached maximum value at the concentration of 100 μg/ml (Fig. 1A and B). Moreover, κ-ca3000 + CP displayed significantly higher protection effect (89.3% for HaCaT cells and 82.1% for MEF cells) than either κ-ca3000 (48.8% for HaCaT cells and 43.6% for MEF cells) or CP (37.8% for HaCaT cells and 34.8% for MEF cells) at their maximum efficient concentration of 100 μg/ml.

In addition, as shown in Fig. 1C and D, κ-ca3000 + CP also displayed higher protective effect (50.2% for HaCaT cells and 46.8% for MEF cells) than either κ-ca3000 (42.3% for HaCaT cells and 38.3% for MEF cells) or CP (36.6% for HaCaT cells and 34.8% for MEF cells) at their maximum efficient concentration of 100 μg/ml. However, the survival rates of the both two cells suggested the protective effects of κ-ca3000 and κ-ca3000 + CP treatment after UV-irradiation were significantly lower than that of the pretreated groups. In other words, the complex κ-ca3000 + CP has better preventive effect than its therapeutic effect.

In conclusion, the complex κ-ca3000 + CP had higher preventive effect than its original independent molecules against UV-irradiation, which suggesting that the protective effect of CP could be enhanced by cross-linked with oligosaccharide.

Fig. 2. Flow cytometric analysis of the subG1 peak of HaCaT cells. The evaluation of DNA content of non-irradiated control HaCaT cells (A), UV-irradiated cells without treatment (B), and UV-irradiated HaCaT cells pretreated with κ-ca3000 + CP (C) were performed by PI-staining. Quantitative analysis of the percentage of cells in pre-G1 phase was also performed (D). Values are means ± S.D. (n = 3). Significance: *p < 0.05, **p < 0.01 vs. UV model group.
3.3. Effect of κ-ca3000 + CP on UV-induced apoptosis

3.3.1. Effect of κ-ca3000 + CP on the subG1 peak in HaCaT cells

It has been reported that UV-irradiation induced cell apoptosis may be mediated by regulation of the cell cycle [19], we therefore examined the effect of κ-ca3000 + CP on cell cycle perturbation by flow cytometric analysis. As shown in Fig. 2B, UV-irradiation induced a distinct subG1 peak, which represents the population of apoptotic cells. κ-ca3000 + CP administration (100 µg/ml) before UV-irradiation could significantly decrease the proportion of HaCaT cells by 34.8% in the subG1 phase (see Fig. 2C).

3.3.2. Effect of κ-ca3000 + CP on the DNA-fragmentation in HaCaT cells

The terminal deoxynucleotidyl transferase (TdT) dUTP nick end labeling (TUNEL) stain is widely used for measuring apoptosis in HaCaT cells exposed to UV-irradiation, as well as in other cell types [28]. PI is used to stain both apoptotic and non-apoptotic cells red. Fluorescein-12-UTP incorporation results in localized green fluorescence within the nucleus of apoptotic cells only. As shown in Fig. 3, κ-ca3000 + CP administration (100 µg/ml) before UV-irradiation led to fewer breaking of DNA than that of UV model group as indicated by the significantly decreased green fluorescence intensity. The results suggest that κ-ca3000 + CP can effectively suppress the apoptosis induced by UV-irradiation.

3.3.3. Effect of κ-ca3000 + CP on the cleavage of PARP in HaCaT cells

Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme activated by binding to a single- or double-strand break of DNA. At the onset of apoptosis, the 116 kDa active form of PARP is proteolyzed to produce two inactive polypeptides of 85–89 and 24–29 kDa [29]. UV-irradiation resulted in an appreciable cleavage of PARP. As shown in Fig. 4, κ-ca3000 + CP treatment significantly decreased the 89 kDa cleavage induced by UV-irradiation at its concentration of 100 µg/ml. That is to say, κ-ca3000 + CP may have an anti-apoptotic role against UV-irradiation.

3.4. ELISA determinations of MMP-1 modulation and collagen I synthesis

Quantitative and qualitative changes in dermal photoaging can be indicated by several morphological and biochemical damage of collagen I, which is the major structural component of the dermis [30]. To estimate the effect of κ-ca3000 + CP and its independent original molecules on skin photoaging, we measured the effects of κ-ca3000, CP and κ-ca3000 + CP on collagen I production and the level of MMP-1 by ELISA assay. As shown in Fig. 5A, the ability to stimulate the production of collagen I was found to be in the following order: κ-ca3000 + CP > κ-ca3000 > CP. The same trend was observed with respect to the inhibition effect of the three compounds on MMP-1 expression (Fig. 5B). Thus, κ-ca3000 + CP could more effectively enhance the production of collagen I and attenu-
ate the expression of MMP-1 at the maximum efficient concentration of 100 μg/ml compared to its independent original molecules.

3.5. Effect of κ-ca3000 + CP on UV-induced activation of MAPK signaling pathways

In skin photaging, UV-irradiation induces the activation of MAPK signaling cascade through phosphorylation of MAPK proteins (ERK, JNK, and p38 kinase), which can lead to skin cell damage [30]. In our experiments, we also observed that the phosphorylation of ERK1/2, JNK, and p38 proteins were increased significantly in UV-irradiated keratinocytes within 2 h over the non-UV-treated control groups. Thus, we investigated the effect of κ-ca3000 + CP on the activation of MAPK signaling pathways by western blot analysis. The results showed that treatment with κ-ca3000 + CP alone at its maximum efficient concentration of 100 μg/ml could significantly influence the phosphorylation of MAPK proteins in UV-treated cells. As shown in Fig. 6, JNK and p38 activation in UV-irradiated keratinocytes markedly decreased by treatment with κ-ca3000 + CP. In addition, there was a conspicuous decrease of phosphorylation of ERK in κ-ca3000 + CP group that occurred 2 h after UV radiation (Fig. 6).

3.6. Intracellular antioxidant activity assay

Choi et al. [31] and Briganti et al. [32] confirmed that UV-irradiation could induce ROS generation in keratinocytes at the dose of 5 J/cm² UVA and 15 mJ/cm² UVB. The accumulation of ROS was most evident 1 h after UV exposure and gradually decreased with prolonged incubation time. To determine the influence of κ-ca3000 + CP on ROS generation, fluorescent probe DCFH-DA was used. In the presence of ROS, DCFH-DA was subsequently transferred to DCF and emitted a green fluorescent signal which could be detected by Flow Cytometer (Becton Dickinson, Vantage, USA) [32]. As shown in Fig. 7, the flow cytometric analysis showed that the mean fluorescence intensity, i.e. the intracellular ROS production, increased about 1.3–1.5 fold in UV-treated cells compared to untreated control cells. However, the increase of intracellular ROS production was significantly inhibited by pretreatment with κ-ca3000 + CP at its maximum efficient concentration of 100 μg/ml in UV-irradiated HaCaT cells (see Fig. 7). The observation suggested that κ-ca3000 + CP might have antioxidant activity by reducing intracellular ROS production in UV-irradiated HaCaT cells.

4. Discussion

Protein–polysaccharide interactions received attention initially due to the fundamental interest and practical significance of this system in the food industry [33]. As the development of biological materials, the collagen protein–marine polysaccharide complexes that confer unique structural and novel properties have been extensively studied and applied in tissue engineering such as burn dressing, tissue scaffold and artificial skin. However, only few studies are available that relate to the applications and novel properties of collagen peptide–marine oligosaccharide complexes. As the strong antioxidants and green biomaterials, both κ-ca3000 and CP showed potential protective effects against UV radiation and offered possibility to produce complexes that can confer special properties. In this study, we firstly tested protective effects of the complex κ-ca3000 + CP against UV radiation. Irradiation of UV with certain level of intensity causes a series of photodamage. High dose of UV radiation can induce massive apoptosis while photoaging can occur after only a minimal dose of UV
In photo-aged skin, the decrease of collagen production is one of the characteristic features. It is generally known that matrix-degrading metalloproteinase (MMP) family related to degradation of collagen in the skin dermis results in photoaging which is characterized by loss of skin tone and resilience, and deep wrinkles [35]. Thus we examined the cell viability as well as the levels of MMPs.

Fig. 6. The effect of κ-ca 3000 + CP on UV-induced activation of MAPKs. At 2 h after UV exposure, total protein samples were extracted from the irradiated cells and the amount of MAPKs were determined by western blotting. The levels of phosphorylated ERK1/2, JNK, and p38 were quantified by densitometric scanning. The ratios of phosphorylated ERK1 (42 kDa), JNK, p38 to total ERK1 (42 kDa), JNK, p38 were calculated respectively. Values are means ± S.D. (n = 3). Significance: * p < 0.05, ** p < 0.01 vs. UV model group.
MMP-1 and collagen I of UV-irradiated HaCaT and MEF cells. Shown in Figs. 1 and 5, κ-ca3000 + CP (100 μg/ml) administration before UV-irradiation could significantly attenuate UV-induced HaCaT and MEF death, increase collagen I production and decrease the level of MMP-1 of MEF by UV radiation. Moreover, κ-ca3000 + CP showed stronger protection effects than its independent original molecules. That is to say, the protection effects of native molecules could be enhanced by cross-linking and the complex κ-ca3000 + CP could also prevent UV-induced skin cell photoaging and apoptosis.

Rittié and Fisher [36] suggested that the suppression action or the prompt repair of UV-induced DNA damage was the marker as a first line of defense against skin cell apoptosis, which could lead to acute skin injury. Shown in Figs. 2 and 3, the treatment of κ-ca3000 + CP led to fewer DNA breaking and reduced 1.6 fold of the percentage of subG1 population as compared to the non compound treated UV-irradiated group. In addition, the PARP cleavage, a prominent feature of the apoptotic execution phase, was inhibited by pretreatment with κ-ca3000 + CP (see Fig. 4). These results indicated that κ-ca3000 + CP possessed the first-line defensive effect against skin photodamage due to the suppression of UV-induced DNA damage.

Many researches proved that the interdependence between UV-caused skin aging and cell apoptosis [34–36]. UV can up-regulate the expression of MMPs and induce apoptosis through the activation of MAPKs signaling protein kinases, including JNK, p38 and ERK, which are important targets affected by ROS levels in cells [36]. Activation of MAPKs can lead to AP-1 complex formation – a regulator of MMP-1 expression. In addition, sustained activation of JNK and p38 produced by UV-irradiation can send a proapoptotic signal and activate the main executors of the apoptotic process [30,35,36]. However, some reports indicated that transient activation of ERK by UV was mainly responsible for skin cell proliferation and differentiation [19]. In this study, we found that κ-ca3000 + CP could significantly affect ERK, JNK and p38 activation in UV-irradiated cells. These results suggested that the inhibition of JNK and p38 activation as well as the enhancement of ERK activation by κ-ca3000 + CP contribute to reversing UV-induced cell damage. That is to say, κ-ca3000 + CP might protect skin cells against UV-irradiation by inhibiting UV-induced activation of the MAPK pathways.

One of the primary reasons that lead to photodamage is the increase of reactive oxygen species (ROS) within the cells after UV-irradiation. ROS can directly damage cellular DNA as well as indirectly regulate MAPKs signaling pathway related to photoaging and apoptosis [37]. Flow cytometric analysis showed that intracellular ROS generation was attenuated by κ-ca3000 + CP treatment. Since κ-ca3000 + CP was able to quench ROS, its suppression of UV-induced MMP-1 expression and DNA damage was expected. The results suggested that κ-ca3000 + CP prevents skin cell injury by UV-irradiation at least partly occurred through its antioxidant effect.

All the results suggested that the antioxidant property of κ-ca3000 + CP can attenuate UV-induced skin photodamage by modulating MMP-1 and collagen I expression, as well as suppressing cell apoptosis through the MAPKs signaling pathways.

5. Conclusion

In conclusion, the results demonstrated that the protective effects of native molecules against UV-induced cell damage could be enhanced by cross-linking and the complex κ-ca3000 + CP could be used for preventing UV-induced photoaging. Intracellular ROS scavenging ability of κ-ca3000 + CP can protect skin cells against UV-induced degradation of collagen I and exert anti-apoptosis effect by regulating MAPKs signaling pathway. Taken together, we provided the first evidence that the peptide–oligosaccharide complex κ-ca3000 + CP has very good photo-protective activities, which enable design and application of the peptide–oligosaccharide complex in cosmetic and pharmaceutical industries.

Conflict of interest

None declared.

Acknowledgements

The authors would like to thank Prof. GuangLi Yu, Prof. Wen-Gong Yu and Dr. Xia Zhao from the Institute of Marine Drug and Food, Ocean University of China for their helpful assistance in this experiment. We also thank M.S. Ping Liu for her generous help in

Please cite this article in press as: S.-W. Ren et al., Protective effects of κ-ca3000 + CP against ultraviolet-induced damage in HaCaT and MEF cells, J. Photochem. Photobiol. B: Biol. (2010), doi:10.1016/j.jphotobiol.2010.06.007
the experiment. This work was supported by a grant from the National High Technology Research and Development Program of China 863 Program Grant (2001AA620405).

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