Effects of topical application of patchouli alcohol on the UV-induced skin photoaging in mice

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A B S T R A C T

Ultraviolet (UV) irradiation, known to generate reactive oxygen species (ROS) excessively and elicit inflammatory response, is a potent inducer for skin photoaging. Overproduction of ROS in conjunction with the resulting inflammation stimulate the over-expression of matrix metalloproteinases (MMPs), which in turn causes degradation of extracellular matrix, leading finally to coarse wrinkling, dryness, and laxity of the skin. In this study, patchouli alcohol (PA, C15H26O), an active chemical ingredient reputed for its capability of scavenging and anti-inflammatory properties, was investigated for its anti-photoaging action using a mouse model whose dorsal skin was depilated. The dorsal skin areas of six-week-old mice were smeared with PA solution or vehicle, followed by UV irradiation for nine consecutive weeks. Protective effects of PA were evaluated macroscopically and histologically, as well as by assaying the antioxidant enzymes (SOD, GSH-Px) activities, the contents of inflammatory factors (IL-10, IL-6, TNF-α), and the levels of MMP-1 and MMP-3. Our findings amply demonstrated that PA significantly accelerated the recovery of the UV-induced skin lesions, evidently through anti-oxidant and anti-inflammatory action, as well as down-regulation of the MMP-1 and MMP-3 expression.

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1. Introduction

As the outer tissue and the largest organ of the human body, the skin is prone to be affected by environmental factors. It has been well known that among the many harmful environmental factors, solar ultraviolet (UV) light is the most noxious, and repeated UV exposure could result in cutaneous oxidative stress and inflammation, which act in concert to cause premature skin aging, a phenomenon known as photoaging. Photoaging is clinically characterized by coarse wrinkles, solar scars, roughness, dryness, laxity and pigmentation (Barone et al., 2008; Chung, 2003) and is histologically characterized by disintegration of elastic fibers, degradation of collagen and thickened epidermal thickness (Yaar and Gilchrest, 2007). Numerous studies for the past several decades have shown that UV-induced damages to the extracellular matrix (ECM) is initiated by generation of reactive oxygen species (ROS) and various inflammatory factors (Masaki, 2010). ROS is one of the most crucial factors of UV-induced photoaging, owing not only to its capability of damaging biological macromolecules such as DNA, carbohydrates, lipids and proteins, but also to its ability to decrease the activities of antioxidant enzymes in the skin, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Kong et al., 2013; Scharffetter-Kochanek et al., 2000). In addition, UV-induced oxidative stress, a result of excessive accumulation of ROS, activates mitogen-activated protein kinase (MAPK) signaling transduction pathway, resulting in the activation of nuclear transcription factor and activator protein-1, with a consequence of releasing inflammatory cytokines such as IL-10, IL-6 and TNF-α (Latreille et al., 2012). Furthermore, excessive ROS together with inflammatory cytokines enhance the expression and activation of different matrix-metalloproteinases (MMPs).

MMPs, a large family of structurally related zinc-dependent endopeptidases, are responsible for the degradation of a wide variety of extracellular matrix (ECM) in the connective tissues (Ho et al., 2005; Rabe et al., 2006). Among them, MMP-1 (interstitial
collagenase) initiates the degradation of types I and III fibrillar collagens (Pillai et al., 2005), while MMP-3 (stromelysin-1) activates proMMP-1 and further degrades the collagen fragments (Rittié and Fisher, 2002). In short, the over-expression of MMPs as a result of ROS generation causes collagen and elastin degradation which is a crucial step leading to the formation of coarse wrinkles and sagging skin. Based on the underlying mechanism of photoaging, it is believed that antioxidant and anti-inflammatory agents can be utilized to attenuate this skin aging process.

The past few decades have witnessed an upsurging interest in the use of herbal medicine and natural products for aging diseases due to their antioxidant and anti-inflammatory properties in addition to their amicable safety profile. Pogostemonis Herba is the dried aerial of Pogostemon cablin (Blanco) Bentham. (Labiatae) that is native to some tropical regions of Asia including China, Indonesia and Malaysia (Liu et al., 2009; Zheng et al., 2006). It has been widely used in traditional Chinese medicine to cure exogenous fever, headache (Zhao et al., 2005), hypotension (Blank et al., 2011), allergy (Suo et al., 2007), thirst (Blank et al., 2011), ache (Lu et al., 2011), dysentery (Liu et al., 2012a), diarrhea (China Pharmacopoeia Committee, 2010; Zhao et al., 2005) and inflammation (Li et al., 2012a; Li et al., 2011; Xian et al., 2011) for centuries. Chemically, it contains bioactive substances such as cytotoxic chalcones, antitumorogenic flavonoids and sesquiterpenes. Among them, sesquiterpenes, the major active sesquiterpene found in Pogostemonis Herba, is largely responsible for the pharmacological activities of this herb.

Our previous in vivo and in vitro studies have revealed that PA possesses strong anti-inflammatory action which is attributable to its ability in suppressing mRNA expression of inflammatory mediators (Li et al., 2012b; Li et al., 2011). Although in recent years, PA has attracted significant research attention in aging protection due to its anti-inflammatory and antioxidant activities, whether it also possesses anti-photoaging effect has not been studied. In the present study, we aimed to evaluate the protective effect of PA on the UV-induced photoaged mice by examining various antioxidant and anti-inflammatory parameters.

2. Materials and methods

2.1. Materials

PA was isolated and purified from Pogostemonis Herba using methods described in our previous studies (Li et al., 2012b; Li et al., 2011). The chemical identity of PA used in the present study (Fig. 1) was confirmed by comparing its spectral data (MS, 1H, and 13C NMR) with the NIST database (Guan et al., 1994; Li et al., 2012b). Moreover, the purity of PA was found to be >98% based on gas chromatographic analysis. PA was used after dissolution in propylene glycol–ethanol (8:2).

Commercial kits used to detect the SOD, GSH-Px activities and MDA, Hyp, protein content were purchased from Jiancheng Institute of Biotechnology (Nanjing, China). In addition, the contents of TNF-α, IL-6, IL-10 (ELISA, eBioscience, Inc. San Diego, CA, USA) and MMP-1 and MMP-3 (ELISA, CUSABIO, Inc. Wuhan, Hubei, China) were measured by enzyme-linked immunosorbent assay. All other chemicals and reagents used in present study were of analytical grade.

2.2. Animals

Female KM mice (18–22 g) were obtained from the Animal Center of Guangzhou University of Chinese Medicine (GZUCM). All experimental procedures were carried out in accordance with the standard guidelines for the care of animals that were approved by the Committee for Animal Care and Use at GZUCM with reference to the European Community guidelines and the regulations of the National Institute of Health of the USA. Mice were housed at a temperature of 23 ± 2 °C and humidity of 55 ± 10% specific pathogen-free environment with a 12 h light/dark cycle and given free access to standard laboratory diet and water. Prior to the start of the experiment, mice were acclimatized for at least one week.

2.3. UV irradiation and PA treatment

According to Table 1, mice were randomly divided into seven groups of 9 mice each after acclimatization. Before the experiment, dorsal skins of mice, except those in the normal control group, were denuded using a lady shaver on the dorsal skin area (2.5 × 3 cm2).

To establish the photoaging model, an array of three UVB lamps, with an emission spectrum between 285 and 350 nm (peak at 310–315 nm), surrounded by five UVA lamps (Waldmann UV800, Germany) emitting exclusively UVA in the range of 320–400 nm (peak at 365 nm), were used as the UV source. Mice in the stainless steel irradiation chamber were subjected to UV irradiation keeping the distance at 30 cm from UV lamps 5 times weekly sparring Wednesdays and Sundays for nine consecutive weeks. The initial dose of UV was set at 100 mJ/cm2 which was defined as the minimal erythemal dose (MED) detected by a Waldmann UV meter (Waldmann Lichttechnik GmbH, Germany) for the first week. Then intensity of UV was increase by 1 MED per week until week 4, and then 4 MED was kept constant for the remaining period of exposure, yielding a total dose of 15 J/cm2 (Kong et al., 2013; Kwon et al., 2013). The non-irradiated control group was treated identically except the UV lamp was switched off.

2.4. Macroscopic evaluation of dorsal skin

The dorsal skins of mice were photographed weekly under anesthesia by diethyl ether inhalation. The photo damage was graded by visual scores according to the grading scale shown in Table 2 by an observer who was blind to the grouping (0 for normal skin while 6 for severely photo-damaged skin) (Bissett et al., 1987; Kong et al., 2013).

2.5. Skin elasticity test

To test skin elasticity, pinch test was carried out weekly in accordance with the modified protocol described by Agrawal and Kaur (2010); Tsukahara et al. (2005). Briefly, the midline of the dorsal skin of the anesthetized mouse was picked up with fingers to a degree that its feet just lightly touched the table. Pinch was subsequently released and the skin recovery time was calculated immediately.

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**Fig. 1.** Chemical structure of patchouli alcohol.
2.6. Histological examination

At the end of the experiment period, mice were sacrificed by cervical dislocation and dorsal skin specimens harvested freshly and fixed in 10% neutral buffered formalin, followed by being embedded in paraffin and sectioned at 5 μm. The degree of skin structure alteration and elastosis were assessed microscopically using Haematoxylin–eosin (H&E) staining (Levy and Barlow, 1989; Uhm et al., 2010) and Gomori’s aldehyde fuchsin staining as described previously (Kong et al., 2013; Proctor and Horobin, 1983).

To quantify epidermal thickness following UV exposure, it was measured at 10 randomly selected locations per slide using an optical microscope (Leica DMLB) with 200 × magnification. Each specimen was photographed under a Leica DC 300 camera. Histological alterations were evaluated and quantified through the image analysis program Image J 1.36 (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) (Gaspar and Maia Campos, 2003; Ouhtit et al., 2000).

2.7. Determination of SOD, GSH-Px and MDA in the skin

Skin samples were harvested from the sacrificed mice and the skin tissue (0.4 g) was homogenized (10,000 rpm, 20 s) with Ultra Turrax (T18 Basic, IKA) in 9 volumes of cold normal saline (4 °C) to obtain the 10% skin tissue homogenate. Before centrifugation, 0.15 ml of the skin tissue homogenate was taken out for the MDA assay and the rest was centrifuged at 3000 × g for 20 min at 4 °C. The total supernatant was used for SOD and GSH-Px measurements and protein content determination according to the manufacturer’s protocols.

2.8. Determination of IL-6, IL-10, TNF-α and MMP-1, MMP-3 in the skin

Another skin tissue (0.4 g) was homogenized (10,000 rpm, 20 s) with Ultra Turrax (T18 Basic, IKA) in 9 volumes of PBS (4 °C) to afford the 10% skin tissue homogenate. The undissolved pellet was removed by centrifugation at 3000 × g for 20 min at 4 °C, and the total supernatant was saved for the subsequent assays. Secreted IL-10, IL-6, TNF-α and MMP-1, MMP-3 were estimated using ELISA kits and protein content was determined as per the manufacturer’s instructions.

2.9. Total collagen content determination

Hydroxyproline (Hyp) is the almost exclusive amino acid of collagen which accounts for approximately 13.4 ± 0.24% in mammalian collagen (Li, 2006; Oxlund et al., 2010). Collagen content of the skin samples can be determined through measuring the amount of Hyp (Neuman and Logan, 1950). The samples were hydrolyzed in 6.0 M HCl, and analyzed for total Hyp content by the Hyp kit according to the manufacturer’s instructions (Jiancheng).

2.10. Statistical analysis

Data were expressed as mean ± SD. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett’s test in order to detect inter-group differences. A value of p < 0.05 was considered to be statistically significant. All analyses were performed using SPSS (version 17.0).

3. Results

3.1. PA ameliorated visual appearance of the photoaged skin

Macroscopic appearances of the skin of the mice at the last week of the experiment period are shown in Fig. 2. During the experiment period, the non-irradiated group (SC) did not exhibit any UV-induced skin damages such as erythema, deep wrinkles and pachulosis, but showed age-related slight wrinkles (Fig. 2A). In contrast, after five weeks of UV exposure, slight erythema and coarse wrinkles were formed in the MC and the VC groups. Nine weeks of UV exposure led to severe wrinkling with deep furrows and laxity in the skin of the MC group; and 5 out of 9 (55.6%) mice even displayed severe erythema, desquamation and scaling (visual scoring: 6). The skin appearance in the VC group was similar to that of the MC group. Thick and deep furrows, and leathery appearance was distinct; and 7 out of 9 (77.8%) VC mice showed flesh-colored skin lesions, while the other two displayed deep wrinkles (visual scoring: 5).

On the other hand, topical administration of PA inhibited the formation of UV-induced erythema, roughness and deep wrinkles in a dose-dependent manner. As shown in Fig. 2A, PA-H group mice exhibited healthy skin with smoothness and only shallow wrinkles. In PA-M group, 5 mice showed slight wrinkles and no erythema, while in PA-L group, 2 mice displayed slight sunburn.

There was no significant difference between the MC and the VC groups in terms of scoring, but both were significantly higher than that in the SC group. However, scores of PA groups were markedly decreased compared to the VC group, indicating that PA could effectively prevent the UV-induced skin damages.

Table 2

Grading scale for evaluation of photoaging.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Evaluation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Smoothness without any wrinkles; fine striations running the length of the body</td>
</tr>
<tr>
<td>1</td>
<td>Fine striations</td>
</tr>
<tr>
<td>2</td>
<td>A few shallow wrinkles; disappearance of all fine striations</td>
</tr>
<tr>
<td>3</td>
<td>Shallow wrinkles across the dorsal skin</td>
</tr>
<tr>
<td>4</td>
<td>Deep and coarse wrinkles with laxity</td>
</tr>
<tr>
<td>5</td>
<td>Increased deep wrinkles</td>
</tr>
<tr>
<td>6</td>
<td>Surface accompanied with severe wrinkles; development of lesions</td>
</tr>
</tbody>
</table>
3.2. PA enhanced skin elasticity in pinch test

To quantify the dorsal skin elasticity of the mice, pinch test was performed weekly and the photographs of mouse dorsal skin after being stretched were illustrated in Fig. 3A. The time of mouse skin to regain its initial shape after deformation was significantly longer in the MC and VC groups than that in the non-irradiated group (SC group). However, there was no significant difference within the MC and VC groups, indicating that vehicle solution had no effect on this indicator. As depicted in Fig. 3B, treatment with different concentrations of PA showed significant reduction in the recovery time (all \( p < 0.05 \) vs. VC group), especially for PA-M and PA-H groups, and the recovery time of which was decreased by 23.4% and 25.8%, respectively.

3.3. PA diminished the UV-induced epidermal thickening

Epidermal thickness as a histological feature of photoaged skin can be used as one of the quantitative parameters to reflect UV caused skin damage, since thickened epidermis can contribute to skin roughness. (Athar et al., 2004; Fujii et al., 2012; Kwon et al., 2013; Pillai et al., 2005; Tanaka et al., 2013; Urikura et al., 2011). As shown in Fig. 4, there was no significant difference in the epidermal thickness either between MC and VC groups, or between NC and SC groups, indicating that the hair removal procedure and the vehicle treatment had no effect on this indicator. The results also showed that repeated UV irradiation could significantly increase the epidermal thickness of MC and VC mice, which was 2.24 and 2.17 times of SC mice (27.14 \( \mu m \)), respectively (all \( p < 0.05 \) vs. SC group). However, in the mice treated with PA, the epidermal thickness was markedly decreased to 47.9 \( \mu m \), 41.2 \( \mu m \) and 37.3 \( \mu m \) in PA-L, PA-M and PA-H, respectively (all \( p < 0.05 \) vs. VC group). These results revealed that PA may have a potential inhibitory effect on skin thickening.

3.4. PA prevented the UV-induced skin structure damage

The pathological process of photoaging stems from a multitude of epidermal and dermal photo damages. Based on the H&E staining, regular skin layers in the NC and SC group were clearly visible, including normal epidermis covered by thin layer of stratum corneum, and completed dermis with well organized hair follicles.
attached by clusters of sebaceous glands. As shown in Fig. 5, either in the NC or SC groups, wavy dermal–epidermal junction (DEJ), which is a narrow wall of wave-like cells between the epidermis and dermis and is essential to the stability of the skin (Khavkin and Ellis, 2011), could be clearly observed. In the superficial dermis, collagen bundles interwoven closely and arranged orderly. Moreover, elastic fibers (as shown in Fig. 6), manifested as deep purple fine line samples, were slender and branched with a homogeneous distribution. Besides, inflammatory infiltrations were not observed in or underneath the skin dermis of the mice in these two groups.

In response to UV irradiation, prominent features of photoaged skin were evidently exhibited in the MC and VC groups. Histologically, epidermal changes in the mouse skin featured the marked thickening of epidermis with flattening of the DEJ and the disappearance of the dermal papillae (Fan et al., 2013). Moreover, UV irradiation induced abnormal keratinization and a greater transformation of the stratum corneum (Fig. 5 MC a). In dermis, as shown in Fig. 5 MC b, tangled and degraded collagen fibers, manifested as a red deposit, could be clearly visible, and the density of these fibers was dramatically decreased. Likewise, elastic fibers, as shown in Fig. 6 MC & VC, fractured into a short, thickened and twisted formation, some of which even collapsed and lost their distribution. Furthermore, in consistent with the previous reports, mice in MC and VC groups displayed skin lesions involving hemorrhage (Fig. 5 MC b) and such lesions also recruited inflammatory infiltration in the entire dermis (Fig. 5 MC c) (Agrawal and Kaur, 2010).

However, topical pretreatment with PA (1.2–2.4 mg/mouse) produced significant protective effects on the UV-induced skin structure damages. The skin of the mice in the PA-H group revealed well-preserved regular epidermis and dermis. Epidermal thickening was significantly inhibited, and the thickness of stratum
corneum was lessened. Moreover, the flattening DEJ was ameliorated compared with the MC mice. In addition, the upper portion of the dermis showed an orderly distribution of hair follicles. In the dermal layer, there was an orderly arrangement of collagen and elastic fibers, of which the thickness and distribution were uniformed. Moreover, inflammatory infiltration could not be seen in these mice skins.

As shown in Fig. 5, PA at the dose of 1.2 mg/mouse (PA-M group) also displayed a protective effect similar to that of 2.4 mg/mouse (PA-H group). Firstly, epidermal thickness was diminished. Secondly, some mice even exhibited interdigitation between the dermal papillae and the epidermal rete ridges. Furthermore, the dermis displayed regularly distributed collagen and elastic fibers, and diffuse inflammation was not observed in this group. Besides, PA at the dose of 0.6 mg/mouse (PA-L group) could also protect the skin from photo damages, though the thickness of epidermis was just slightly thinner than that of the VC group and the dermis exhibited incomplete collagen and elastic fibers. In addition, inflammatory infiltration was absent in and underneath the dermis.

3.5. PA restored the activities of the antioxidant enzymes

The effects of PA on oxidative stress in the skin tissue were examined by measuring the activities of SOD and GSH-Px. As depicted in Table 3, the activities of these two antioxidant enzymes remained unaltered either between the NC and the SC groups or between the MC and the VC groups, indicating that hair shaving operation and vehicle treatment did not affect these indicators. In contrast, UV irradiation significantly decreased the activities of SOD and GSH-Px in the MC and VC groups, respectively (all \( p < 0.05 \) vs. SC group). Nevertheless, the decreased activities of SOD and GSH-Px were markedly reversed by the application of PA when compared with that in the VC group, although GSH-Px activity was not significantly different between the PA-M and the VC groups. Taken together, these results unequivocally indicated that PA was able to reduce oxidative stress in the skin through enhancement of antioxidant enzyme activities.

3.6. PA inhibited MDA production

MDA is a well-known biomarker for lipid peroxidation. As shown in Table 3, there was no significant difference in the MDA level between the NC and the SC groups, and between the MC and the VC groups. However, the MDA content was dramatically elevated by about 3 folds in the MC group (\( p < 0.05 \) vs. SC group). PA treatment caused a 30.0% reduction when compared to the VC group (all \( p < 0.05 \)), and there was no significant difference among the three doses of PA.
3.7. PA suppressed the production of inflammatory cytokines

In this study, the production of inflammatory cytokines, such as IL-6, IL-10 and TNF-α, was examined since they were closely associated with cellular damage and molecular integrity of the skin. Results depicted in Table 3 showed no significant difference in the levels of IL-6, IL-10 and TNF-α either between the NC and the SC groups or between the MC and the VC groups, revealing that hair removal procedure and vehicle treatment did not stimulate the production of these inflammatory cytokines. In comparison with the SC groups, the levels of IL-6, IL-10 and TNF-α were significantly up-regulated in the MC group. However, pretreatment of PA (at the doses of 1.2–2.4 mg/mouse) was able to significantly attenuate the production of IL-6, IL-10 and TNF-α when compared with the VC group. These data was indicative that PA could effectively suppress the release of the inflammatory cytokines as a result of UV exposure.

3.8. PA reversed UV-induced increase of MMPs’ content

MMPs (especially MMP-1 and MMP-3) have been known to play important roles in the degradation of skin collagen. In this study, we measured the expression of MMP-1 and MMP-3 to elucidate the molecular mechanisms underlying protective effects of PA on the UV-induced collagen degradation. As shown in Table 3, the contents of MMP-1 and MMP-3 were not significantly different between the NC and the SC groups, as well as between the MC and the VC groups. However, the expression of MMP-1 and MMP-3 was much higher in the MC group than that in the SC group. When treated with 2.4 mg/mouse of PA, the contents of MMP-1 and MMP-3 were notably attenuated by about 24.6% and 39.3% respectively (all $p < 0.05$ vs. VC group), but no significant difference was found between the PA-L group and the VC group. These data suggested that PA could markedly inhibit the increase of MMP-1 and MMP-3 expression induced by repeated UV exposure, thereby preventing the degradation of skin collagen.

3.9. PA enhanced skin collagen content

Collagen content is the main component that renders the skin smoothness. No obvious difference in collagen content was found either between the NC and the SC groups or between the MC and the VC groups, while collagen contents of the MC and the VC groups were significantly reduced when compared to the SC group ($p < 0.05$). However, pretreatment with 1.2–2.4 mg/mouse of PA was able to significantly increase the collagen content (all $p < 0.05$) when compared with the VC group (Table 3). These results demonstrated that UV radiation contributed to a reduction in collagen content, whereas the reduction of collagen was markedly reversed by pretreatment of PA.
4. Discussion

Photoaged skin, often attributed to chronic UV irradiation (Matsumura and Ananthaswamy, 2004), is characterized by an increase in skin thickness, a reduction in skin elasticity and formation of wrinkles. These observable changes in the skin appearance are associated with a reduction in the collagen and elastic fibers as a result of increased production of MMPs. It has been demonstrated that the production of MMPs is closely linked to the UV-induced inflammation and the resultant accumulation of ROS (Fisher et al., 2002; Urikura et al., 2011).

PA, a known anti-oxidative and anti-inflammatory agent (Chantous et al., 2010; Li et al., 2011; Murakami, 2009), is purported to be a promising agent for the prevention of photoaging.

Table 3 Effect of PA on various parameters of photoaged mice skin.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mgprot)</th>
<th>GSH-Px (U/mgprot)</th>
<th>MDA (nmol/mgprot)</th>
<th>TNF-α (pg/mgprot)</th>
<th>IL-6 (pg/mgprot)</th>
<th>IL-10 (pg/mgprot)</th>
<th>MMP1 (ng/mgprot)</th>
<th>MMP-3 (ng/mgprot)</th>
<th>Collagen (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>40.87 ± 5.18</td>
<td>45.85 ± 8.36</td>
<td>1.07 ± 0.36</td>
<td>20.01 ± 8.93</td>
<td>19.72 ± 5.02</td>
<td>202.85 ± 83.47</td>
<td>7.64 ± 1.55</td>
<td>14.55 ± 1.89</td>
<td>18.40 ± 1.89</td>
</tr>
<tr>
<td>SC</td>
<td>39.23 ± 10.76</td>
<td>43.86 ± 9.05</td>
<td>1.13 ± 0.31</td>
<td>21.58 ± 9.86</td>
<td>21.14 ± 8.84</td>
<td>234.84 ± 72.36</td>
<td>9.51 ± 1.89</td>
<td>14.79 ± 3.10</td>
<td>17.03 ± 2.38</td>
</tr>
<tr>
<td>MC</td>
<td>31.74 ± 6.63</td>
<td>32.84 ± 9.73</td>
<td>3.17 ± 0.55</td>
<td>37.35 ± 9.03</td>
<td>41.33 ± 6.05</td>
<td>462.27 ± 141.86</td>
<td>13.24 ± 2.04</td>
<td>20.13 ± 5.04</td>
<td>10.10 ± 4.34</td>
</tr>
<tr>
<td>VC</td>
<td>30.91 ± 5.49</td>
<td>30.97 ± 3.42</td>
<td>3.33 ± 0.70</td>
<td>33.82 ± 4.65</td>
<td>38.15 ± 9.07</td>
<td>474.06 ± 166.22</td>
<td>13.75 ± 2.65</td>
<td>21.12 ± 3.30</td>
<td>9.95 ± 4.09</td>
</tr>
<tr>
<td>PA-L</td>
<td>41.19 ± 4.87</td>
<td>42.75 ± 12.28</td>
<td>2.42 ± 0.56</td>
<td>23.59 ± 8.69</td>
<td>32.80 ± 9.22</td>
<td>390.64 ± 95.68</td>
<td>14.64 ± 3.64</td>
<td>19.93 ± 5.79</td>
<td>9.67 ± 5.81</td>
</tr>
<tr>
<td>PA-M</td>
<td>38.79 ± 4.32</td>
<td>35.59 ± 5.65</td>
<td>2.35 ± 0.97</td>
<td>24.39 ± 6.75</td>
<td>27.18 ± 6.16</td>
<td>319.35 ± 128.94</td>
<td>11.92 ± 2.82</td>
<td>16.52 ± 2.35</td>
<td>11.49 ± 6.70</td>
</tr>
<tr>
<td>PA-H</td>
<td>49.29 ± 8.26</td>
<td>44.17 ± 14.02</td>
<td>2.23 ± 0.83</td>
<td>22.77 ± 8.89</td>
<td>26.19 ± 9.86</td>
<td>315.63 ± 121.60</td>
<td>10.37 ± 2.31</td>
<td>12.82 ± 1.50</td>
<td>15.16 ± 1.74</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 9 mice per group.

A One unit of SOD activity was defined as the amount of the enzyme inhibiting the oxidation by 50%.
B One unit of glutathione peroxidase is defined as the amount of the enzyme leading 1 μmol GSH oxidized per min.
C MDA content was expressed as nmol per mg protein.
D TNF-α was expressed as pg per mg protein.
E IL-6 was expressed as pg per mg protein.
F IL-10 was expressed as pg per mg protein.
G MMP-1 content was expressed as ng per mg protein.
H MMP-3 content was expressed as ng per mg protein.
I Collagen content was expressed as μg per mg skin.
* significantly different from vehicle control group (p < 0.05).
# significantly different from sham group (p < 0.05).
In the present study, the protective efficacy of PA in photoaged mouse skin was evaluated and our data revealed for the first time that this compound not only significantly attenuated the UV-induced wrinkle formation, saggy skin, but also inhibited the extra-cellular matrix degradation and epidermal thickening by enhancing the activities of various antioxidant enzymes, suppressing inflammatory response and inhibiting the production of MMP-1 and MMP-3.

We found that the UV-induced decrease in collagen content, which was accompanied with the degradation of elastic fibers, was an important early event leading to wrinkled appearance and saggy skin. Our findings are consistent with other’s observations (Fan et al., 2013; Hou et al., 2009). Collagen, comprising 75% of the dry weight of the dermis, is mainly responsible for maintaining the tensile strength and mechanical resistance of the skin (Fuji et al., 2012; Hou et al., 2012). Besides collagen, elastic fibers also contribute to skin plasticity and resilience (Afnan et al., 2012). However, these two major components of ECM can be directly degraded by matrix metalloproteinase (MMPs), the important photochemical products of UV irradiation (Chiang et al., 2011). MMPs are a family of zinc binding endopeptidases that contribute to the destruction of the basement membrane structure (Dong et al., 2008). MMP-1, an interstitial collagenase, is one of the most important MMPs responsible for degradation of numerous types of collagen, notably types I and III collagen (Kim et al., 2012). Moreover, it can suppress the transcription of the type I collagen gene (You et al., 2013). Once collagen is cleaved by MMP-1, it can be further degraded by MMP-3, which not only activates proMMP-1 but also degrades the non-collagenous components and elastic fibers of the dermal ECM (Rittié and Fisher, 2002).

Our results revealed that both PA-M and PA-H could conspicuously suppress MMP-3 secretion, subsequently result in the reduction of MMP-1. The present results also showed that the production of MMP-1 was markedly suppressed by PA-H.

Moreover, in accordance with the measurement of MMPs, we also found in the histological study that mice after PA treatment exhibited an accumulation of elastic fibers and an augmentation of collagen density. These findings were further confirmed by Hyp content determination. Based on the above findings, we believe that PA is a potent MMP inhibitor eliciting effective inhibition in the secretion of MMP-3, subsequently downregulated MMP-1 production. Our findings are in good agreement with that of CHIANG et al. (2011).

Production of MMPs is intimately regulated by both ROS and inflammatory cytokines (Chan et al., 2003). It has been shown that ROS can activate cell surface receptors and affect intracellular signaling through stimulating the stress-associated receptors MAPK p38 and c-Jun amino terminal kinase-JNK 2 (Yaar and Gilchrest, 2007). Activation of kinases subsequently enhances the activity of transcription factors such as nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1), and finally upregulates the expression of MMPs (Bickers and Athar, 2006). Under physiological condition, endogenous antioxidant enzymes such as SOD and GSH-Px are secreted in sufficient amount to scavenge the cellular ROS by catalyzing them into O2− and H2O2, thereby keeping the expression of MMPs at a physiologically low level (Scharffetter-Kochanek et al., 2000). However, UV radiation can dramatically elevate the ROS generation, which eventually overwhelms the action of endogenous antioxidant enzymes, aggravates MMPs expression and lipid peroxidation, with a consequence of causing serious wrinkling and sagging appearance in the skin (Pillai et al., 2005), an observation which was amply demonstrated in our present study. Moreover, our data also showed that PA could reverse the UV-mediated reduction of SOD and GSH-Px activities and attenuate the MDA production, a generally accepted index for determining the degree of lipid peroxidation. Considering that three doses of PA could significantly elevate the activity of SOD and suppress the MDA production, we therefore conclude that PA has a potent effect on scavenging superoxide anion radicals (O2−) and protecting the skin from lipid peroxidation. Moreover, PA could suppress the expression of MMP-1 and MMP-3, possibly through its protective effect on antioxidant enzyme system. This conclusion is congruent with previous studies showing that PA had potent free radicals scavenge activity (Ghanous et al., 2010; Ho et al., 2005; Murakami, 2009).

Besides the UV-induced ROS, ROS-activated AP-1 and NF-kB can also enhance the synthesis of inflammatory cytokines and promote the infiltration and activation of inflammatory cells in the skin (Kwon et al., 2013; Pillai et al., 2005). The results of H&E staining which revealed numerous inflammatory cells within and underneath the dermis were in consistent with macroscopic appearance of the photoaged mouse skin. Moreover, inflammatory cytokines IL-6, IL-10 and TNF-α themselves are potent NF-kB activators that form an auto-activating loop (Chung et al., 2009), resulting in activating the MMPs and contributing to the loss of interstitial collagen in the photoaged cutaneous (Kim et al., 2005; Pillai et al., 2005). Previous experimental evidence also suggests that IL-10 plays a crucial role in the UVB radiation-induced immune suppression (Enk et al., 1993), and that IL-6 is the common inflammatory marker closely associated with chronic diseases and the proinflammatory properties (Maggio et al., 2006). In addition, TNF-α has a role in the recruitment of cells to areas of inflammation by altering adhesion molecule expression (Chae and Kwak, 2003; Thornfeldt, 2008). Therefore, topical application of an anti-inflammatory agent may protect the skin from UV damage.

In our previous study, we have already found that PA markedly decreased the production of inflammatory mediators by suppressing their mRNA expression in vivo and in vitro (Li et al., 2012b; Li et al., 2011). In the present study, PA also exhibited anti-inflammatory activity by inhibiting the production of IL-6, IL-10 and TNF-α, which was reflected in the recovery of skin smoothness and the elimination of inflammatory cells gathering. We found that PA at 0.6–2.4 mg/mouse could suppress TNF-α secretion, and believe that PA has a unique effect on TNF pathways. The specific pathways such as NF-kB and JNK pathway will remain an area of investigation in our future study.

To sum up, this is the first study to demonstrate the protective effects of PA, a naturally-occurring sesquiterpene monomer, on the UV-induced photoaged mouse skin. Evidence was presented in this paper that PA dramatically suppressed the UV-induced overproduction of MMP-1 and MMP-3 through enhancing the activities of antioxidant enzymes and inhibiting the production of inflammatory markers, which finally reversed the UV-mediated collagen and matrix destruction. Although the specific regulatory pathway of PA remains to be elucidated, we believe that PA has a strong potential to be developed into a therapeutic and cosmetic product against skin photaging.

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