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Anti-photoaging effects of chitosan oligosaccharide in ultraviolet-irradiated hairless mouse skin

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ABSTRACT

Skin photoaging (SP) is a premature skin-aging damage after repeated exposure to ultraviolet (UV) radiation, mainly characterized by oxidative stress and inflammatory disequilibrium, which makes skin show the typical symptoms of photoaging such as coarse wrinkling, dryness, irregular pigmentation and laxity. Chitosan oligosaccharide (COS), a natural polysaccharide with good humectant property, is the depolymerized product of chitosan with various biological activities, among which the antioxidant and anti-inflammatory effects have been frequently reported in recent years. However, no existing *invivo* study indicates whether COS has direct protective effect on UV-induced SP. In the current research, we investigated the potential preventive effect of COS against UV-caused damage in hairless mouse dorsal skin. The data showed that COS, by topical application after each UV-radiation for 10 weeks, effectively inhibited the undesirable changes on the skin induced by UV. To be specific, COS obviously alleviated the macroscopic and histopathological damages of mice skin, *via* mitigating the disrupted collagenous fibers, as well as improving the relative content of type I collagen and the amount of total collagen. Furthermore, COS effectively inhibited the levels of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, and markedly improved the activities of antioxidant enzymes (SOD, GSH-Px, CAT), as well as the content of skin hydroxyproline and moisture. These findings demonstrated that this natural polysaccharide attenuated UV-induced SP, at least in part, by virtue of favorable regulation of antioxidant and anti-inflammatory status, which presumably worked in concert to maintain the morphology and level of dermal collagen.

1. Introduction

It is well-accepted that the most important determinant of skin photoaging (SP) is repeated exposure of skin to ultraviolet (UV) radiation, actually referring to UVB (290–320 nm), which is absorbed mainly by the epidermis, and UVA (320–400 nm), which enters the deep dermis (Battie et al., 2014; Berneburg et al., 2000; Sanches Silveira and Myaki Pedroso, 2014; Wu et al., 2011). Considerable research has shown that SP is a progressive and complex deterioration, which is mainly characterized by overproduction of reactive oxygen species (ROS) induced by UV. Excessive ROS directly result in cutaneous oxidative stress and pro-inflammatory responses, which then cause inflammatory disequilibrium, ultimately leading to protein oxidation, lipid peroxidation, the mitochondria and DNA damages, and even apoptosis of skin cells (Herrling et al., 2006; Jenkins, 2002; Ju et al., 2017; Moriwaki and Takahashi, 2008). Meanwhile, ROS have an additional role in skin aging by directly or indirectly activating the enzymes, such as matrix metalloproteinases (MMPs), hyaluronidase and elastase, which then disrupt collagenous and elastic fibers, and diminish the moisture of the skin, finally resulting in the degradation of extracellular matrix (ECM), an essential structural framework of skin (Kahari and Saarialho-Kere, 1997; Vayalil et al., 2004). Upon degradation of ECM, typical symptoms of photoaging such as coarse wrinkles, dryness, loss of elasticity, hyperkeratosis and even a variety of benign, premalignant and malignant neoplasms occur (Im et al., 2016; Moriwaki and Takahashi, 2008). Currently, close attention has been paid to natural products due to their amicable safety profile and potentially beneficial biological activities, and searching natural ingredients with antioxidant, anti-inflammatory and humectant property for preventing and/or treating this skin photo-aging damage becomes a new hot spot in skin care research. Chitosan oligosaccharide (COS), consisting of 2–10 β-1,4-linked D-glucosamine units, is the depolymerized product of

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chitosan. Compared with chitosan, COS, the sole oligosaccharide with a positive charge in nature, possesses superior biological activities such as antimicrobial, antioxidant, lowering of blood cholesterol and pressure (Kim and Rajapakse, 2005), in addition to its lower molecular weight and higher water-solubility (Thadathil and Velappan, 2014; Xu et al., 2017). Previous studies have revealed that COS is biocompatible, non-toxic and absorbable, as well as possessing various biological functions such as anti-bacterial, neuro-protective, anti-diabetic, anti-tumoral and anti-hypoglycemic properties, which grant it potential in pharmaceutical applications (Azuma et al., 2015; Muanprasat and Chatsudthipong, 2017; Thadathil and Velappan, 2014). Furthermore, there are also reports displaying that COS can increase the antioxidant capacity, maintain a favorable redox balance, and possess anti-inflammatory activity by regulating signaling pathways (Fang et al., 2015; Muanprasat and Chatsudthipong, 2017). For instance, COS can attenuate oxidative stress-induced retinal damages in rats (Fang et al., 2013), protect mice from LPS challenge (Li et al., 2014), prevent retinal I/R injury in rats and accelerate weaned pig growth mainly by maintaining the activities of anti-oxidative enzymes and inhibiting the activation of NF-κB (Fang et al., 2015; Wan et al., 2017), as well as regulating mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Ma et al., 2011).

In addition, the function of COS in skin has attracted increasing attention in recent years for its skin-permeable and moisturizing abilities. COS, which has *N*-acetyl-d-glucosamine, an important component of skin ECM, can provide glycone to modify O-linked b-Nacetylglucosamine (O-Glc NAc), and then promote kin tissue repair (Runager et al., 2014). Notably, it has been reported that COS can markedly suppress the UVB-induced increase in mRNA and protein expression of matrix metalloproteinases-1 (MMP-1) and matrix metalloproteinases-3 (MMP-3) in skin fibroblasts (Zheng and Deng, 2013). However, to our best knowledge, no existing *invivo* study indicates the direct protective effect of COS against UV-induced SP. For the above bioactivities and structural analysis of COS, whether COS has anti-SP effect is clearly worth exploring. Thus, in the current research, we endeavored to investigate the potential effect of COS on UV-induced damage in hairless mouse dorsal skin and explore the possible underlying mechanisms. Our result for the first time suggested that COS possessed appreciable protective effect against UV-induced SP, which was intimately associated with its antioxidative and anti-inflammatory properties. These findings provided a pioneering pharmacological basis for the anti-SP effect of COS and suggested that COS might be a potential candidate for the therapy of SP.

2. Materials and methods

2.1. Materials and chemicals

COS (average molecular weight ≤ 1000 Da, degree of deacetylation ≥ 90%, water-soluble), a white powder, was purchased from Beijing Zhong Tai He technology (ZTH tech, Beijing, China). A certain amount of COS was weighed accurately, then mixed and added slowly into the double-distilled water at room temperature to prepare the clear and transparent solution with three different concentrations (200, 100 and 50 mg/ml). Based on the weak UV absorption of COS at 290–400 nm (as displayed in Fig.1), topical application after each UV-radiation was employed to investigate its therapeutic effect.

Commercially available kits for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), mouse hydroxyproline and protein concentration were provided by Nanjin Jiancheng Bioengineering Institute (Nanjing, China). Tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β levels were measured by enzyme-linked immunosorbent assay (ELISA) kits, obtained from eBioscience (San Diego, CA, USA). All other chemicals and reagents used in the study were of analytical grade.

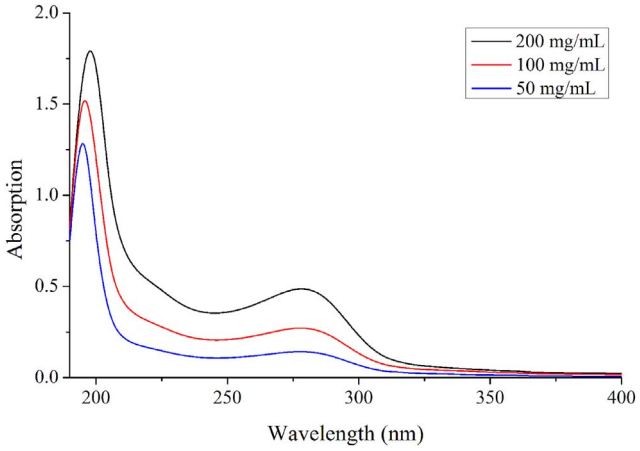

Fig. 1. The UV absorption spectra of chitosan oligosaccharide (COS).

2.2. Animals and experimental design

Seven-week-old female hairless BALB/c mice (n = 12), weighing approximately 16 g (animal quality inspection No.44007200042574), were received from the Center of Laboratory Animal Science of Guangdong Province (certified No. SCXK (Yue) 2013–0002). Animal experiments were performed according to the institutional guidelines of the experimental animal center of Guangdong Medical University for the care and use of laboratory animals (certificate number: SYXK (Yue) 2015–0147). The mice were housed in a temperature and humidity controlled room (23 ± 2 °C, 55 ± 10%) with a 12 h light/12 h darkness cycle (without any ultraviolet emission), and given food and water *ad libitum* during the experimental period.

After a week of acclimation, the hairless mice were randomly divided into five groups of 6 mice per group according to previous literatures (Im et al., 2016; Jimbo et al., 2015; Kong et al., 2015; Lee et al., 2014): Normal control group (without UV irradiation but with double-distilled water treatment); Model group (UV irradiation with double-distilled water treatment); COS-L group, COS-M group and COS-H group (UV irradiation with 50 mg/ml, 100 mg/ml and 200 mg/ml COS treatment, respectively). For mice used for topical application, 100 μl were applied to each mouse dorsal skin every time after UV-radiation. The dorsal treated skin area of mice was carefully wiped with soft absorbent cotton soaked in distilled water, and then wiped with dry cotton before each UV-radiation to remove any remaining COS.

2.3. UV irradiation

UV irradiation was performed according to the method previously reported by our group (Kong et al., 2015). The UV source was supplied by an array of UVB lamps and UVA lamps (Waldmann UV800, Germany), the absorbance peaks of which were at 310–315 nm and 365 nm, respectively. Irradiance was measured using a Waldmann UV meter (Waldmann Lichttechnik GmbH, Germany), and the minimal erythemal dose (MED) of the dorsal mouse skin was determined to be approximately 55 mJ/cm^2. UV radiation was applied to the dorsal skin of hairless mice 5 times/week for 10 weeks. The starting dose of UV radiation was 1 MED during the first week, and the dose was increased weekly by 1 MED until reaching 4 MED, which was maintained until 10 weeks.

2.4. Macroscopic evaluation

Under anesthesia by diethyl ether, the visible diversification of dorsal skin in hairless mice was examined and photographed at the end of the 10th week, the grade of which was determined by an observer who was blind to the groups according to the evaluation criteria