Anti-wrinkle Effect of PLA₂-free Bee Venom against UVB-irradiated Human Skin Cells

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ABSTRACT

The use of bee venom (Apis mellifera L., BV) occasionally causes side effects such as inflammation and allergic reactions in the recipients. Several case reports also suggested the treatment of BV has some limitations in its clinical uses, due to the occurrence of dermal necrosis and anaphylactic reactions. It is generally understood that bee venom allergy is mainly the result of its allergic component, phospholipase A₂ (PLA₂). The present study was aimed to generate PLA₂-free bee venom (PBV) and evaluate its efficacy as skin care and cosmetic preparation, comparing with original bee venom (BV). Our results showed that both BV and PBV exhibited significant protective effects in UVB-irradiated human keratinocyte (HaCaT) and human dermal fibroblast (HDF) cells and they also induced type I collagen synthesis in UVB-irradiated HDF cells except BV at 3 μg/ml. Furthermore, BV and PBV showed the inhibition of UVB-stimulated matrix metalloproteinase-1 (MMP-1), a major collagen degrading enzyme in skin. However, BV, unlike PBV, exhibited strong cytotoxicities in skin cells (both HaCaT and HDF) at its working concentrations of anti-wrinkle effect. The underlying cell signaling mechanisms of anti-wrinkle effects of BV and PBV were demonstrated by the activation of ERK1/2, and p38. Conclusively, PBV appears to be the bee venom of choice with less cytotoxicity and higher efficacy on UVB-irradiated skin cells in comparison with original bee venom (BV). Therefore, PBV can better be used as a cosmetic ingredient exhibiting excellent anti-wrinkle effect against photoaging than original BV.

Key words - Anti-wrinkle effect, Bee venom, Phospholipase A₂ free bee venom, Ultraviolet B
1. Introduction

Skin aging is a natural process of chronological changes of our skin. On the other hand, premature aging is an unnatural aging process and mostly caused by a long-term exposure to Ultraviolet (UV) irradiation which is probably due to increased outdoor lifestyle and wide use of tanning devices for cosmetic purposes. It is referred to as photoaging and is characterized by rough wrinkles, epidermal thickness, increased levels of MMPs, inflammation and collagen degradation (Fineschi et al., 2007; Gilchres, 1989). The UV spectrum is generally divided into three types, UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). UVC is the most powerful energy, but it is almost completely absorbed by the ozone layer. Whereas UVA and UVB reach the Earth’s surface. UVB is a lesser extent energy reaching to the earth’s surface, it is 500-800 times more harmful than UVA (Pillai et al., 2005). Several studies demonstrated that UVB is the most dangerous light causing skin cancer in experimental animals and inducing DNA damage (Yoshino et al., 2002; Tzung and Rünger, 1998). In addition, UVB irradiation is responsible for epidermal thickness and degradation of extracellular matrix (ECM), leading to damage of skin tissue integrity, formation of wrinkle and inflammation (Rittié and Fisher, 2002). Therefore, the protection of skin from UVB irradiation may contribute to prevent the processes of wrinkle formation, photoaging and inflammatory reactions of the skin.

Matrix metalloproteases (MMPs) are usually secreted from fibroblasts and keratinocytes, the major target cells of UVB irradiation in skin. MMPs degrade ECM that plays a pivotal role in the maintenance of dermal skin layers (Kim et al., 2004). Skin is mainly composed of collagen (70-80% dry weight) and the overproductions of MMPs can induce abnormal degradation of ECM, resulting in loss of elasticity, integrity and wrinkle formation in skin. In addition, continuous exposure of UVB may generate inflammatory mediators, resulting in the activation of MMPs. Accordingly, the inhibition of the MMPs can be one of the best strategies for the protection and prevention of wrinkle formation against UVB irradiation.

In recent years, several natural reagents have been reported to protect UVB-mediated skin damage and their application on skin care products has been increasing (Bae et al., 2008; Afaq and Mukhtar, 2006). Carnosic acid, a phenolic diterpene, inhibits the UVB-induced MMPs in human skin fibroblasts and keratinocytes through suppression of ROS production and ERK/AP-1 activation (Park et al., 2013). The green tea polyphenols have been also reported to protect UV-induced oxidative damage and modulate MMPs expression at low concentrations without tachyphylaxis in a human study, proposing its therapeutic potential as photoaging inhibitor (Vayalil et al., 2004).

Bee venom (BV) has caught people’s attention as a cosmetic ingredient due to its protective, antibacterial and anti-inflammatory effects on skin (Han et al., 2013). Previous studies have demonstrated that BV reduces protein levels as well as mRNA expressions of UVB-stimulated MMP-1 and -3 and it also protect damage of UVB-irradiated human dermal fibroblasts (Han et al., 2007). However, sometimes the BV-containing products have been accused of having adverse effects (erythema and allergy reaction) that have created a profound disturbance in its utilization. For the safe use of BV in skin care products, we prepared PLA₂-free bee venom (PBV) by removing PLA₂ from natural BV using ultrafiltration and investigated its therapeutic potentials in comparison with natural BV, with the focus on its beneficial