Oral Supplementation with Cocoa Extract Reduces UVB-Induced Wrinkles in Hairless Mouse Skin

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Cacao beans contain various bioactive phytochemicals that could modify the pathogeneses of certain diseases. Here, we report that oral administration of cacao powder (CP) attenuates UVB-induced skin wrinkling by the regulation of genes involved in dermal matrix production and maintenance. Transcriptome analysis revealed that 788 genes are down- or upregulated in the CP supplemented group, compared with the UVB-irradiated mouse skin controls. Among the differentially expressed genes, cathepsin G and serpin B6c play important roles in UVB-induced skin wrinkle formation. Gene regulatory network analysis also identified several candidate regulators responsible for the protective effects of CP supplementation against UVB-induced skin damage. CP also elicited antiwrinkle effects via inhibition of UVB-induced matrix metalloproteinases-1 expression in both the human skin equivalent model and human dermal fibroblasts. Inhibition of UVB-induced activator protein-1 via CP supplementation is likely to affect the expression of matrix metalloproteinases-1. CP supplementation also downregulates the expression of cathepsin G in human dermal fibroblasts. 5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone, a major in vivo metabolite of CP, showed effects similar to CP supplementation. These results suggest that cacao extract may offer a protective effect against photoaging by inhibiting the breakdown of dermal matrix, which leads to an overall reduction in wrinkle formation.

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INTRODUCTION

Many natural products are known to influence the development of skin structures and its biological functions. Cacao beans have the antioxidant capacity higher than the capacity provided by green teas and red wine (Lee et al., 2003; Subhashini et al., 2010). The antioxidant activity of cacao can modify the pathogeneses of a different spectrum of diseases, including the cardiovascular diseases, cancer, and other chronic conditions (Park et al., 2014). Recent studies have demonstrated the beneficial effects of cacao consumption are associated with human health, especially with the improved condition of the skin (Park et al., 2014; Scapagnini et al., 2014). Cacao provides positive effects on the skin structure and the dermal microcirculation (Katz et al., 2011; Neukam et al., 2007), and its topical preparations are able to protect the skin from the oxidative damages arising from UV radiation (Katz et al., 2011). Besides the photoprotection against UVB-induced erythema, long-term ingestion of cacao also ameliorates aberrant skin conditions by increasing the blood flow to the cutaneous and subcutaneous tissues to increase the skin density and hydration (Heinrich et al., 2006). Our previous studies have shown that cacao inhibits skin cancer growth and skin inflammation both in vitro and in vivo (Kang et al., 2008; Kim et al., 2010; Lee et al., 2006). Although there is accumulating evidence that cacao consumption can improve the skin health, the molecular mechanisms responsible for these beneficial effects have not been thoroughly investigated.

Skin wrinkling is a typical characteristic of photoaging that results from chronic exposure to solar UV radiation. Repeated exposure to UV light decreases procollagen production and breaks down collagen fibers. The process is partially due to the overexpression of matrix

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Abbreviations: AP-1, activator protein; CP, cacao powder; DEGs, differentially expressed genes; DHPV, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone; ECM, extracellular matrix; GRN, Gene Regulatory Network; HDF, human dermal fibroblasts; HSE, human skin equivalent; MEdD, minimal edema dose; MMP, matrix metalloproteinases; Nrf2, NF-E2-related factor 2; TFs, transcription factors

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metalloproteinases (MMP) (Fisher et al., 1997; Ichihashi et al., 2009; Xu and Fisher, 2005). Our previous studies demonstrated that cathepsin G regulates MMP expression and UVB-induced skin photoaging (Son et al., 2009, 2012). Cathepsins comprise a family of serine proteases whose members are classified into A, B, C, D, E, G, H, and L groups, according to their substrate specificities (Son et al., 2009). Cathepsins B, D, K, and G may act as biomarkers in photoaged human skin (Zheng et al., 2011). Cathepsin G is a single 30-kDa polypeptide released by the neutrophils and the UVA-irradiated normal human fibroblasts (Son et al., 2009). Inhibitors of cathepsin G may be useful for the prevention of UVB-induced photoaging because they could ameliorate the extracellular matrix (ECM) damage and MMP upregulation (Son et al., 2012). Serpin b6 is a member of the superfamily of serine protease inhibitors known as serpins. Serpins bind with serine proteases involved in inflammatory processes, coagulation, fibrinolysis, tumorigenesis, and apoptosis. The association of serpin b6 with cathepsin G has been postulated to inhibit cathepsin G activity (Scott et al., 2007).

In this study, we first examined the protective effect of CP on UVB-induced wrinkle formation in hairless mice, and then we showed gene expression profiles using RNA sequencing analysis in comparison with several other well-known food materials used to modify skin health (Cho et al., 2007; Marini et al., 2012). To better investigate the antiphotoaging effects of CP and the implications for clinical settings, we measured its effects using a human dermal fibroblasts (HDF) and human skin equivalent (HSE) model.

RESULTS

Oral administration of CP reduces UVB-induced wrinkle formation and prevents UVB-induced collagen degradation

To investigate the effect of CP on wrinkle formation, the dorsal skins of hairless mice were exposed to UVB with low and high concentrations of CP (CL, 39.1 mg/kg, CH, 156.3 mg/kg) and pycnogenol (Pyc, 625 mg/kg) for 8 weeks as described (Figure 1a). UVB-induced wrinkle formation was markedly reduced in the CP-administered groups (Figure 1b). Quantification of skin wrinkle severity through the assessment of the area of wrinkling (Figure 1c) and visual wrinkle grade (Figure 1d) confirmed a significant decrease in wrinkle formation in the CP groups. We then stained the skin samples of the mice with Masson's trichrome staining to observe the effect of CP on amorphous collagens of the skin (Figure 1e). Collagen levels gradually recovered in the CP groups to an extent greater than the UVB-irradiated group (Figure 1e, Supplementary Figure S1 online). The physical aesthetics of the CP groups were similar or superior to those of the pycnogenol-treated group (Figure 1c–e). Taken together, these results suggest that oral administration of CP reduces UVB-induced wrinkle formation and prevents UVB-induced collagen degradation.

Expression profiling of differentially expressed genes (DEGs) mediated by CP supplementation and/or UVB-irradiation of mouse skin tissue

To identify genes associated with the UVB-protective effect of CP supplementation in skin, we systematically analyzed the transcriptome from the mice exposed to UVB-irradiation and/or administrated with CP and pycnogenol. The heat map of DEGs in the UVB-irradiated mice indicated that 788 genes were up- or downregulated by at least one concentration of CP supplementation (Figure 1f). Among the 788 DEGs, 156 genes were upregulated by UVB compared with control and downregulated by CH compared with UVB-irradiation (Figure 1g), and 199 genes were downregulated by UVB-IR compared with the controls and upregulated by CH compared with the UVB irradiated group (Figure 1h). Supplementation with CH elicited transcriptomic recovery on the up- and downregulated genes after UVB-IR (Figure 1g and h). Furthermore, CP administration shows more impact on transcriptomic recovery than the recovery induced by pycnogenol (Figure 1g and h), suggesting that CP may be a more potent antiphotoaging agent than pycnogenol.

Expression patterns of genes associated with antiphotoaging

To further characterize gene expression patterns, we identified the genes associated with antiphotoaging effects using the related gene ontology terms including extracellular matrix disassembly (Figure 2a), cell adhesion (Figure 2b), lipid metabolic process (Figure 2c), and proteinaceous extracellular matrix (Figure 2d and Supplementary Table S1 online). The gel-like ECM is the largest component of the dermal skin layer and comprises a variety of fibrous structural proteins, including collagens, elastin, laminin, and proteoglycans such as dermatan sulfate and hyaluronan (Bradley et al., 2015). Differentially expressed genes in ECM disassembly indicated that CP-fed mice had markedly inverted changes in their UVB-mediated transcriptomes (Figure 2a). CP significantly diminished UVB-induced cathepsin G (Ctsg) expression. Interestingly, the effect of CP on these expression patterns was more significant than that of pycnogenol (Figure 3a). Among the various serpin b6 genes, CP supplementation specifically enhanced the expression of serpin b6c (Figure 3b). These findings suggest that both inhibition of cathepsin G and induction of serpin B6c by CP supplementation may contribute to a protective effect against UVB-induced wrinkle formation. To identify potential mediators of the changes in transcriptome expression patterns, we constructed a Gene Regulatory Network (GRN) analysis composed of the DEGs in Figure 3c, and significantly enriched transcription factors (TFs) obtained from the TF-target relationships derived from the Encyclopedia of DNA Element data (Consortium, 2012; Gerstein et al., 2012) and SignaLink database (Fazekas et al., 2013) (Figure 3d and e). Thus, GRN analysis identifies the mediators involved in the antiphotoaging effects of CP.

CP prevents UVB-induced MMP-1 upregulation in HDF and in HSE layers

To better understand whether the antiwrinkling effects of CP in mice could be relevant for clinical settings, we examined the effect of CP on collagenase (MMP-1) in HDF and HSE layers. CP treatment elicited a decrease in MMP-1 protein expression in a concentration-dependent manner and significantly suppressed the mRNA levels of UVB-induced MMP-1 (Figure 4a and b). Furthermore, CP inhibited UVB-induced activator protein (AP-1) transactivation (Figure 4c). These inhibitory effects arose within a concentration range that did not significantly affect cell viability in the presence of UVB irradiation (Figure 4d). These results suggest that CP may