Food Chemistry 216 (2017) 19–26

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Theobroma cacao extract attenuates the development of Dermatophagoides farinae-induced atopic dermatitis-like symptoms in NC/Nga mice

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ARTICLE INFO

Article history:
Received 25 April 2016
Received in revised form 23 July 2016
Accepted 26 July 2016
Available online 27 July 2016

Keywords:
Atopic dermatitis
Cacao
Immune cell infiltration
Inflammatory cytokines
Inflammatory skin lesions

ABSTRACT

Cacao beans from *Theobroma cacao* are an abundant source of polyphenols, particularly flavonoids. Previous studies demonstrated that cacao flavanols decrease pro-inflammatory cytokines resulting in the alleviation of allergic symptoms. We sought to investigate the effects of cacao extract (CE) on Dermatophagoides farinae extract (DFE)-induced atopic dermatitis (AD)-like symptoms. CE attenuated DFE-induced AD-like symptoms as assessed by skin lesion analyses, dermatitis score, and skin thickness. Histopathological analysis revealed that CE suppressed DFE-induced immune cell infiltration into the skin. These observations occurred concomitantly with the downregulation of inflammatory markers including serum immunoglobulin (Ig) E, chemokine; thymus and activation-regulated chemokine and macrophage-derived chemokine as well as the skin-derived cytokines interleukin (IL)-4, IL-5, and interferon-γ. CE also significantly alleviated transepidermal water loss and increased skin hydration. These results suggest that CE, a natural phytochemical-rich food, has potential therapeutic efficacy for the treatment of AD.

1. Introduction

Atopic dermatitis (AD) is a chronic skin disorder characterized by relapsing skin inflammation, disturbance of skin barrier function, and immunoglobulin (Ig) E-mediated sensitization to allergens (Grewe et al., 1998). AD has become a significant health problem worldwide, and its prevalence has increased nearly three-fold in industrialized countries over the past three 30 years (Bieber, 2008). A recent analysis has estimated that more than one million children across 97 countries currently suffer from the disease (Williams & Flohr, 2006). In mechanistic terms, AD is related to several other allergic disorders including asthma, food allergies, and allergic rhinitis (Leung, Boguniewicz, Howell, Nomura, & Hamid, 2004). AD is also generally thought to be one of the initial stages in the atopic March, a procession from AD to allergic rhinitis and asthma (Bantz, Zhu, & Zheng, 2014). AD arises from a complex interaction between the skin as a functional barrier and the immune system. During the progression of AD, the skin barrier function collapses owing to inflammation and itching, resulting in decreased hydration of the stratum corneum and an increase in transepidermal water loss (TEWL) (Mu, Zhao, Liu, Chang, & Zhang, 2014).

The pathogenesis of AD involves T-helper cell (Th) type 2 immune responses together with the dysregulated interleukin 4 (IL-4), IL-5, and IL-13 production (Soumelis et al., 2002). The influx of Th2 cytokines induces isotype class switching from IgM to IgE (Abril-Gil et al., 2012). Following these Th2 responses, eosinophils and mast cells are recruited toward the inflammatory skin lesions (Mochizuki, Bartels, Mallet, Christophers, & Schroder, 1998). The

Abbreviations: CE, cacao extract; AD, atopic dermatitis; DFE, Dermatophagoides farinae extract; TEWL, transepidermal water loss; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine.

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http://dx.doi.org/10.1016/j.foodchem.2016.07.141
0308-8146/

expression of CC chemokine receptor (CCR) 4 ligands, such as thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) leads to the further recruitment of Th2 lymphocytes to the inflammatory site (Jahnz-Rozyk, Targowski, Paluchowska, Owczarek, & Kucharczyk, 2005). AD lesions frequently exhibit higher populations of infiltrating Th1 cells and cytokines from Th1 cell including interferon (IFN)–γ induce apoptosis of keratinocytes. It aggravates the symptoms of atopic dermatitis (Ong & Leung, 2006).

Recently, therapeutic ways for the treatment of AD have focused on the use of corticosteroids. However, these agents often elicit undesirable side effects including high blood pressure, sickness, vomiting, kidney injuries, and headaches. There are also complications involved when prescribing such treatments to children. Therefore, a considerable unmet need exists for development of safer and more effective AD treatments (Kang et al., 2015; Park et al., 2005).

Chocolate is one of the most widely recognized foods in the world, of which cacao is the essential ingredient (Kim et al., 2014). Cacao is an abundant source of polyphenols, particularly flavonoids, which primarily exist in the form of monomers (epicatechins and catechins), or oligomers (procyanidins) (Rios et al., 2002). Dark chocolate refers to forms that are at least 54% cacao powder, and typically contains high levels of catechins (107–132 mg/kg) and epicatechins (327–502 mg/kg) (Arts, van de Putte, & Hollman, 2000). The polyphenolic structures of these constituents have been shown to exert beneficial effects toward the prevention of various diseases, including inflammation, cancer, cardiovascular disease, and other chronic diseases (Scalbert, Johnson, & Saltmarsh, 2005). Our previous studies showed that cacao extract (CE) inhibits solar ultraviolet induced wrinkle formation in vivo and regular cocoa flavanol consumption had positive effects on facial wrinkles and elasticity In moderately photo-aged women (Kim et al., 2016; Yoon et al., 2016). However, correlation between consumption of cacao extract and the development of AD has not been reported. Therefore, we sought to investigate whether cacao extract (CE) can suppress the development of AD-like symptoms in NC/Nga mice.

2. Materials and methods

2.1. Materials

Three-week-old male NC/Nga mice were provided from SLC Japan (Tokyo, Japan). Dermatophagoides farinae extract (DFE) was purchased from Biostir (Hiroshima, Japan). Tacrolimus was purchased from Astellas Pharma (Tokyo, Japan). All ELISA kits were purchased form R&D Systems (Minneapolis, MN). RNA Isolation Kit were purchased from Ambion Ltd (Huntingdon, Cambridgeshire, UK). PrimeScript™ 1st strand cDNA synthesis Kit was purchased from Takara Bio Inc. (Shiga, Japan). IQ SYBR was purchased from Bio-Rad Laboratories, Hercules, CA. Sodium dodecyl sulfate was purchased from Sigma (St. Louis, MO).

2.2. Preparation of CE and animal diets

Cacao extract (CE) was provided by Barry Callebaut (Lebbeke-Wieze, Belgium). *Theobroma cacao* beans were roasted and ground to make cacao liquor, which was separated from cacao butter, to produce cacao cakes. Cacao cakes were ground and 300 kg of ground cacao was extracted using 2400 L of 70% ethanol at 60 °C for 12 h. The extract was filtered and dried (Kim et al., 2016). CE was produced by grinding the cacao cakes. AIN-93G formulation (Harlan, Spain) was used as the standard diet for the animal study. Previous studies showed that this diet does not comprise detectable concentrations of polyphenols (Ramiro-Puig, Urpi-Sarda et al., 2007). 0.25% and 1% CE diets were made by alteration of the AIN-93G formula by deducting the amount of carbohydrates, proteins, lipids and fiber provided by the related percentage of added CE. Accordingly, the final chow was isoenergetic with the standard diet.

2.3. Characterization of CE

The filtrate (10 μl) was injected into HPLC (Thermo Fisher Scientific, Waltham, MA). For chromatographic analysis (wavelength: 280 nm, solvent gradient: 40 min), C18 column (4.6 × 250 mm) was used. Three compounds were analyzed under these HPLC conditions and they did not have any other ingredients (Fig. 1).

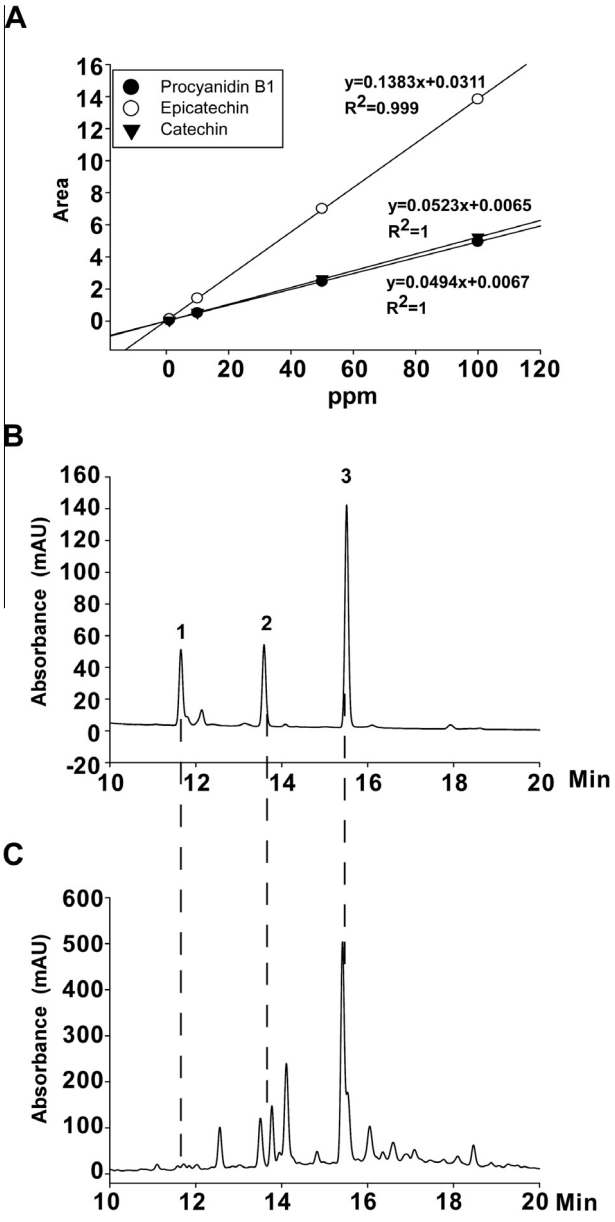

Fig. 1. Characterization of cacao extract (CE) by HPLC. The amount of the active marker (procyanidin B1, catechin, epicatechin) were analyzed using an analytical HPLC with an acetonitrile and water gradient system. The analytes were quantified by UV (280 nm). *A*, Calibration curves of each chemical. *B*, The HPLC spectrum of standard. *C*, The HPLC chromatography of CE. 1: procyanidin B1, 2: catechin, 3: epicatechin.