Journal of Functional Foods 49 (2018) 458–464

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Tomato saponin supplementation ameliorates the development of experimental arthritis by regulating inflammatory responses

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ARTICLE INFO

Keywords:
Collagen-induced arthritis
Esculeogenin A
Inflammatory cytokines
Tomato saponin

ABSTRACT

We evaluated the effect of tomato saponin, containing esculeoside A, a major saponin found in Japanese pink-colored tomato, on collagen-induced arthritis DBA/1J mice. Arthritis scores in collagen-injected mice were markedly lower by tomato saponin supplementation than that of the control throughout the 15-days observation period. Notably, there was a significant negative correlation between the arthritis scores and serum concentrations of esculeogenin A, an aglycon of esculeoside A ($R^2 = 0.84$, $p < 0.05$). Additionally, mRNA expression of pro-inflammatory cytokines in the knee joint were significantly decreased by tomato saponin. Moreover, $CD38^+CD45R^+$ splenocytes were significantly increased by tomato saponin, suggesting the shift to the type II helper T cell (Th2) response. In cultured splenocytes from DBA/1J mice, purified esculeogenin A also significantly suppressed the lipopolysaccharide-induced IL-12p40, Th1-driving component, and pro-inflammatory cytokines. Our results suggest that esculeogenin A potentially ameliorates collagen-induced arthritis by inhibiting the pro-inflammatory cytokines involved in the activation of Th1.

1. Introduction

The tomato plant (*Solanum lycopersicum*) originated in the Andes of South America and is one of the most popular food crops. It is cultivated worldwide and consumed both as a raw fruit and in various processed foods. Lycopene, a carotenoid pigment present in tomato, has been recognized for its functional effects such as its antioxidant response (Di Mascio, Kaiser, & Sies, 1989) and carcinogenic inhibitory effects (Nishino et al., 2002). Other functional components of tomato have been recently reported. Processed tomato products contain 13-oxo-9,11-octadecadienoic acid, which has been demonstrated to improve lipid metabolism (Kim et al., 2012). Tomato saponin is a major group of secondary metabolites in tomato fruits (Iijima et al., 2013). The tomato saponin, α-tomatine, is present in concentrations of up to 500 mg/kg in fresh, immature green tomatoes (Friedman, 2002). α-tomatine and its aglycone form, tomatidine, act as antibiotics toward plant pathogens and as cancer chemotherapeutic agents (Friedman, 2002). Intake of green tomato extract (Choi et al., 2013), α-tomatine (Friedman, Fitch, & Yokoyama, 2000), or tomatidine (Fujiwara et al., 2012) has been shown to affect serum lipid levels. As the color changes from green to red during ripening, the α-tomatine concentration decreases as it is converted into esculeoside A (Nohara et al., 2007). Esculeoside A, which has been characterized as 3-*O*-β-lycotetraosyl (5S,22S,23S,25S)-23-acetoxy-3β,27-dihydroxyspirosolane 27-*O*-β-$_D$-glucopyranoside (Fig. 1A), is abundantly found in the methanolic extracts of cherry and pink tomatoes that are widely cultivated in Japan (Fujiwara et al., 2004). In some tomato species, 79–87% of the total glycoalkaloids are esculeoside A (Friedman, 2002). Esculeoside B, an isomer of esculeoside A, is abundant in red tomatoes such as Italian tomatoes. Cherry tomatoes contain 400–500 mg/kg esculeoside A, which is approximately 21 times their lycopene content (Katsumata et al., 2011). However, the amount of esculeoside A in commercial tomato products is lower than the limit of detection (Manabe et al., 2011) because both the sugar moiety and steroidal backbone of esculeoside A are easily degraded by heating under acidic conditions (approximately a 30% decrease at 100 °C for 30 min) (Katsumata et al., 2011). Esculeoside A is also converted into

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https://doi.org/10.1016/j.jff.2018.09.009
Received 22 March 2018; Received in revised form 9 September 2018; Accepted 9 September 2018

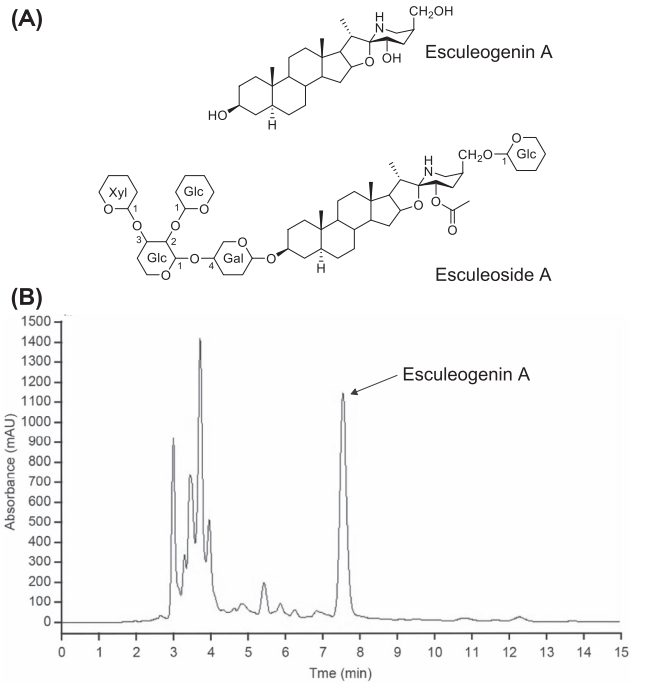

Fig. 1. (A) Structures of esculeogenin A and esculeoside A. (B) HPLC analysis of tomato saponin fraction. Chromatogram was obtained by the monitoring the absorbance at 210 nm. Esculeogenin A was eluted at 7.6 min.

pregnane glycoside in over-ripe fruits. Androstane derivatives, which are probably metabolized via pregnane derivatives from tomato steroidal glycosides, can be detected in urine samples that are collected from subjects who have consumed tomatoes (Nohara et al., 2007). The chemical structure of esculeogenin A (Fig. 1A), an aglycone form of esculeoside A, is similar to that of diosgenin. Diosgenin, also a steroidal sapogenin, is found in wild yam. Diosgenin has various effects on cancer (Raju & Mehta, 2009; Miyoshi et al., 2011), diabetes (McAnuff, et al., 2005), and inflammation (Choi et al., 2010; Hirai et al., 2010). The anti-arteriosclerotic effect of esculeogenin A has already been reported (Fujiwara et al., 2007). Moreover, α-tomatine and tomatidine inhibit nuclear factor-κB (NF-κB) activation (Chiu & Lin, 2008; Lee, Wong, Cheah, & Mustafa, 2011). Therefore, we hypothesized that esculeogenin A would demonstrate anti-inflammatory effects. Collagen-induced arthritis (CIA) is induced by immunizing mice with type II collagen, which is the main component of joints (Trentham, 1982). Anti-type II collagen antibodies drive the activation of antigen-specific T cells that progress the arthritis. Because the destructive inflammation of CIA resembles rheumatoid arthritis (RA) pathogenesis (Williams, 2004), CIA is often used as an animal model of RA. Curcumin, the active principle of spice turmeric (*Curcuma longa*), has been showed anti-inflammatory effects in CIA model by suppressiong production of pro-inflammatory mediators and matrix metalloproteinases (Huang et al., 2013; Moon, Kim, Choi, Park, & Kim, 2010; Mun et al., 2009; Okamoto, Tanaka, Fukui, & Masuzawa, 2011). In addition, the efficacy of curcumin has been observed in human patients with active RA (Chandran & Goel, 2012). In the present study, we investigated the effects of the intake of tomato extract containing esculeogenin A on mice with CIA using curcumin as a positive control.

2. Materials and methods

2.1. Sample preparation

An aqueous tomato extract was prepared from cherry tomatoes without the calyx (4.0 kg) using a previously reported method (Fujiwara et al., 2004). Briefly, cherry tomatoes were smashed with a mixer and filtered. The supernatant (3.5 kg) was spray dried, yielding an aqueous tomato extract (329 g). The components of the aqueous tomato extract are shown in Table S1. To prepare the saponin fraction, 35 g of aqueous tomato extract was dissolved with 400 ml of ultrapure water and filtered. The supernatant was passed through the Diaion HP-20 column (35 × 120 mm, Mitsubishi Chemical Corporation, Tokyo, Japan). The tomato saponin fraction was eluted with 500 ml of methanol and dried using an evaporator, thereby yielding 700 mg of tomato saponin fraction. HPLC analysis (solvent, 45% methanol and 1% acetic acid; flow rate, 1.0 ml/min; detection, 210 nm) was performed to confirm the presence of esculeoside A in the tomato saponin fraction (Fig. 1B). The amounts of esculeoside A present in the tomato saponin fraction were 7.9% (w/w). Esculeoside A was kindly provided by Professor Toshihiro Nohara, Sojo University, Japan. Esculeoside A was boiled under acidic conditions for 1 h, and the boiled sample was then mixed with ethyl acetate and centrifuged. The ethyl acetate layer was harvested and dried. Highly purified esculeogenin A was prepared according to a previous report (Fujiwara et al., 2004).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.09.009.

2.2. Collagen-induced arthritis

Six-week-old male DBA/1J mice were purchased from Charles River Japan (Yokohama, Japan). The mice were housed in socially compatible groups in individually ventilated cages. Mice were given free access to drinking water and a standard CE-2 diet (CLEA Japan, Tokyo, Japan). The room in which the mice were kept was maintained under control conditions of temperature (23 ± 2 °C), humidity (50 ± 10%), and a 12:12 light:dark cycle. The Institutional Animal Care Center Committee at the University of Shizuoka approved all animal sample collection methods (Approval number: 125033). The animal experiments were done in accordance with the “Guidelines for Proper Conduct of Animal Experiments” from the Science Council of Japan and the guidelines of the Animal Care Center Committee at the University of Shizuoka.

After a habituation period, the mice were intracutaneously injected with 40 μl of an emulsion that was composed of equal volumes bovine type II collagen (Chondrex, Redmond, WA, USA) and complete Freund’s adjuvant (Chondrex) in the base of tail. Blood samples were collected from the tail vein on Day −1 (22 days after the first immunization) and Day 13, and the concentration of serum anti-type II collagen antibody was measured using a commercial kit (Chondrex). On Day 0, the mice were divided into three groups (control, n = 21; tomato saponin, n = 20; and curcumin, n = 19) in a manner that normalized the concentration of serum anti-type II collagen antibody among the groups. Subsequently, the mice in each group were resensitized with an intraperitoneal injection (20 μl) of bovine type II collagen. All the mice (housed 4–6 mice/cage) were fed an experimental diet and drinking water *ad libitum* until Day 15 (Fig. 2). The feed of the control, tomato saponin and curcumin groups were AIN-76; AIN-76 supplemented with 1% saponin fraction; and AIN-76 supplemented with 1% curcumin (Wako Pure Chemical Industries, Osaka, Japan), respectively (Table 1). An arthritis score that indicated the development and severity of

Fig. 2. Experimental design. Arrows above the boxes indicate the first (Day −23) and second (Day 0) sensitizations of the mice with type II collagen. The gray square indicates the experimental diet-feeding period. Open triangles indicate the timing of body weight measurement. Body weights were measured every third day. Closed triangles indicate the timing of blood collection for ELISA testing. The arthritis scoring was daily performed.