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Grape seed proanthocyanidin extract has potent anti-arthritic effects on collagen-induced arthritis by modifying the T cell balance

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation of the synovial joints, joint malformations, and disability. The continuous use of conventional anti-inflammatory drugs is associated with severe adverse effects. Grape seed proanthocyanidin extract (GSPE) is considered to have protective effects against several diseases. In this study based on the mouse adjuvant-induced-arthritis (AIA) model, we examined the effects of GSPE on the key mediators of arthritic inflammation, namely T cell subsets, glucocorticoid-induced tumour necrosis factor receptor (GITR) expressing cells, $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells, Th17 cells, Th1/Th2 cytokines, and inflammatory mediator gene expression. We treated BALB/c mice with 25, 50, or 100 mg/kg GSPE or saline daily (14 days) per orally (p.o.) at the onset of AIA. At the peak phase of AIA (day 14), the heparinised whole blood and ankle joints of all groups were collected and tested. GSPE-treated mice showed a substantial reduction in the levels of T cell subsets, GITR-expressing cells, and Th1 cytokines as well as the inflammatory mediators (MCP-1, MIP-2, and ICAM-1) that induce them compared with the vehicle-treated (saline) and arthritic mice. However, GSPE significantly upregulated the number of Tregs and Th2 cytokine producing cell number or it also induced Th17/Treg rebalance and orchestrated various pro-inflammatory and anti-inflammatory cytokines and the gene expression of their mediators that mediate cellular infiltration into the joints. This might, contribute to its anti-arthritic activity. Our results suggest that p.o. treatment with GSPE attenuated AIA in mice might offer a promising alternative/adjunct treatment for RA.

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1. Introduction

The immunogenetics of RA suggest a key role for aberrant pathways of T cell activation in the initiation and/or perpetuation of the disease. Although T-helper (Th) 1, Th2, and Th17 cells are specialised in immunity during viral, parasitic, and other infections, T-regulatory (Treg) cells are dedicated to the control of immune responses and mediate tolerance against harmless non-self- or self-antigens. The differentiation and function of Treg cells require the transcription factor forkhead box p3 (Foxp3), and inducible Treg cells can be generated in the periphery from naïve $CD4^+$ T cells [1]. However, autoimmunity and inflammation are regulated by the balance between Th17 and Treg cells [2]. Niu et al. [3] showed that there is an imbalance in the ratio between Th17 and Tregs, revealing an increase in Th17 and a decrease in Treg cells in RA patients compared with healthy controls. This imbalance plays a critical role in RA progression. Furthermore, Treg cell activation has an essential role in the prevention of autoimmunity [4], and cell-based therapy with Treg cells has the potential to produce durable disease remission in patients with RA.

TGFβ has been detected in RA synovial tissue, and the suppressive effects of synovial fluid have been attributed to its actions [5]. IL-4 and IL-10 were first identified as products of Th2 clones and are cytokines that distinguish Th2 cells from other T cells. In addition, IL-4 and IL-10 inhibit the production of IL-2 and IFN-γ by Thl cells, resulting in the suppression of macrophage activation [6]. IL-4 and IL-10 inhibit the production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF-α, by monocytes and macrophages [7].

Polyphenols, which are abundantly present in vegetables and fruits, are functionally active molecules possessing a novel spectrum of biological, therapeutic, and chemopreventive properties [8,9]. Proanthocyanidins are the most abundant phenolic compounds in grape seeds and exert antibacterial, antiviral, anti-carcinogenic, anti-mutagenic, anti-inflammatory, anti-allergic, and vasodilatory effects

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[8]. Proanthocyanidins are highly bioavailable and provide a significantly greater protection against damage from oxidative stress than vitamin C, vitamin E, and β-carotene [10]. Proanthocyanidins inhibit lipid peroxidation, platelet aggregation, capillary permeability, and fragility and modulate the activity of some enzyme systems, including phospholipase A2, cyclooxygenase, and lipoxygenase [8]. Grape seed proanthocyanidin extract attenuates collagen induced arthritis [11] and differentially regulates Foxp3 regulatory and IL-17 pathogenic T cell in autoimmune arthritis [12]. Based on these results we proved that GSPE modulate Th1/Th2 cells and cytokine balance in functional and molecular levels.

Adjuvant-induced arthritis (AIA) can be induced in BALB/c mice by immunisation with complete Freund's adjuvant (CFA) containing heat-killed mycobacteria, and it shares many of the characteristic features associated with RA [13]. In this study of AIA in mice, we examined the effect of grape seed proanthocyanidin extract (GSPE) on paw oedema and the production of T cell subsets ($CD4^+$, $CD25^+$, and $CD4^+CD25^+$), $Foxp3^+$ Tregs, $CD4^+CD25^+Foxp3^+$ Tregs, $CD4^+$ T cells that secreted intracellular cytokines (IL-2, TNF-α, IFN-γ, IL-17A, and IL-4), and GITR-expressing cells using flow cytometry in heparinised whole blood (Fig. 1). We investigated the effects of GSPE on pro-inflammatory cytokines IL-2, IL-1β, IL-6, IFN-γ, TNF-α, IL17A, and ICAM-1; anti-inflammatory cytokines IL-4, IL-10, IL-13, TGF-β1; and mRNA expression in ankle tissues using reverse transcription real-time PCR (RT-PCR).

2. Materials and methods

2.1. Animals

Female adult BALB/c mice that were 6–7 weeks old and weighed 20–22 g were acquired from the animal house of the College of Pharmacy at King Saud University, Riyadh, Kingdom of Saudi Arabia. These mice were kept at room temperature (22 ± 2 °C) under a 12 h light/dark cycle. The mice were housed in a specific pathogen-free environment and fed standard rodent chow and water ad libitum. All procedures were performed with the approval of the Institutional Animal Care and Use Committee.

2.2. Chemicals

GSPE was obtained from (Spectrum Chemicals Jersey Ave, New Brunswick, USA). The other reagents used for this study were fluorescein isothiocyanate (FITC)-labelled CD4, Foxp3, and IL-17A anti-mouse monoclonal antibodies; allophycocyanin (APC)-labelled CD25, IL-2, IL-4, TNF-α, IFN-γ, and phycoerythrin (PE)-labelled GITR anti-mouse monoclonal antibodies; FcR blocking reagent; Fixation/Permeabilisation Solution (Miltenyi Biotec, Germany); heparin (Sigma-Aldrich, USA); and CFA containing heat-killed mycobacteria (Chondrex, Inc., USA).

2.3. Initiation of AIA and GSPE treatment

BALB/c mice were immunised with a subcutaneous injection of 0.02 ml CFA containing 5 mg/ml of heat-killed mycobacteria [14]. Proanthocyanidin dissolved in saline was administered per orally (p.o.) to four different groups (25, 50, and 100 mg/kg) after immunisation. The mice were treated p.o. with different doses of GSPE (25, 50, and 100 mg/kg) once daily for 2 weeks. The control mice were given saline. Heparinised whole blood and ankle joint samples were collected from all treated and control mice groups two weeks after the injection and ankle joint samples were stored at −70 °C until use.

2.4. Histopathological assessment of ankle joint

The ankle joints were removed at 14 days following AIA injection, fixed for 4 days in 10% formalin, decalcified in decal solution (EDTA) in 5% formic acid embedded in paraffin and sectioned (7 μm). Tissue sections were stained with haematoxylin and eosin (H&E) and were evaluated under a light microscope. Bone destruction, vascular proliferation, synovial hyperplasia and inflammatory cell infiltration were assessed.

2.5. RNA extraction and cDNA synthesis

All extraction procedures were performed on ice using ice-cold reagents. Total RNA from ankle tissue homogenate from each mouse was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA was quantified by measuring the absorbance at

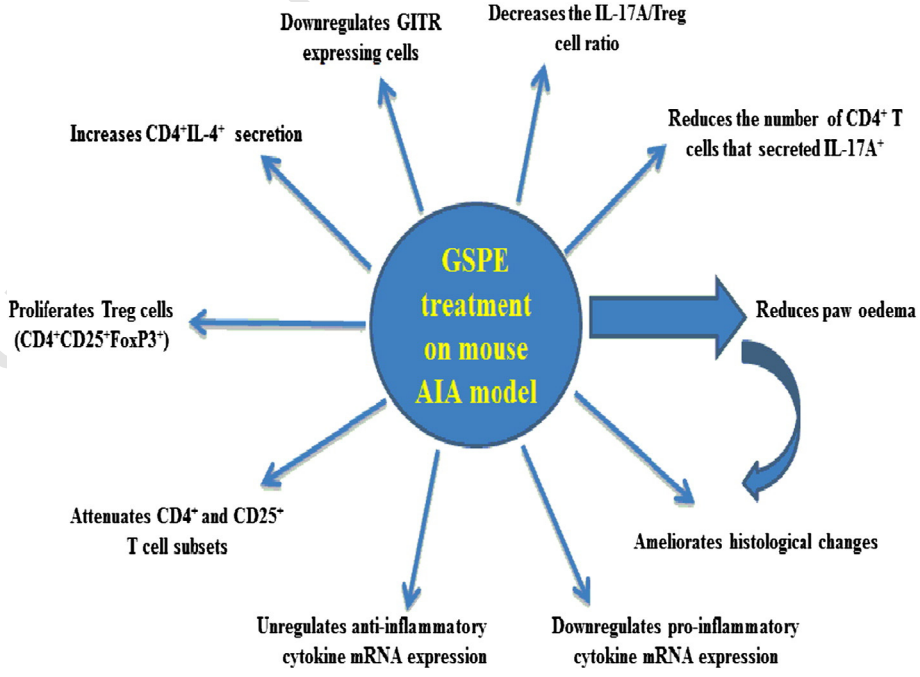

Fig. 1. Schematic diagram to explain the protective mechanism of GSPE treatment (grape seed proanthocyanidin extract) on mouse adjuvant-induced-arthritis (AIA) model.

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