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Gallic acid suppresses inflammation in dextran sodium sulfate-induced colitis in mice: Possible mechanisms

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ABSTRACT

Inflammatory bowel diseases (IBD) encompass at least two forms of intestinal inflammation: Crohn's disease and ulcerative colitis (UC). Both conditions are chronic and inflammatory disorders in the gastrointestinal tract, with an increasing prevalence being associated with the industrialization of nations and in developing countries. Patients with these disorders are 10 to 20 times more likely to develop cancer of the colon. The aim of this study was to characterize the effects of a naturally occurring polyphenol, gallic acid (GA), in an experimental murine model of UC. A significant blunting of weight loss and clinical symptoms was observed in dextran sodium sulfate (DSS)-exposed, GA-treated mice compared with control mice. This effect was associated with a remarkable amelioration of the disruption of the colonic architecture, a significant reduction in colonic myeloperoxidase (MPO) activity, and a decrease in the expression of inflammatory mediators, such as inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and pro-inflammatory cytokines. In addition, GA reduced the activation and nuclear accumulation of p-STAT3^{Y705}, preventing the degradation of the inhibitory protein IκB and inhibiting of the nuclear translocation of p65-NF-κB in colonic mucosa. These findings suggest that GA exerts potentially clinically useful anti-inflammatory effects mediated through the suppression of p65-NF-κB and IL-6/p-STAT3^{Y705} activation.

1. Introduction

Inflammatory bowel diseases (IBD) encompass at least two forms of intestinal inflammation: Crohn's disease and ulcerative colitis (UC). Both types are chronic and inflammatory disorders in the gastrointestinal tract, with an increasing prevalence associated with the industrialization of nations and rising in developing countries [1]. The pathogenesis of IBD remains unclear, but imbalances between pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-1β, IL-6, and IL-12, and anti-inflammatory cytokines, such as IL-4, IL-10, and IL-11, are believed to play a central role in modulating inflammation [2,3]. Patients with these diseases are 10–20 times more likely to develop cancer of the colon or bowel cancer [4,5]. Therapeutic strategies for treating inflammatory bowel disease now focus on the use of anti-inflammatory agents [6], and plant-based remedies play an important role in the therapy of many inflammatory disease conditions, including IBD [7–9].

Signal transducer and activator of transcription (STAT)-3 is a key member of the STAT protein family, which has been shown to play significant roles in cytokine signaling pathways. Under normal circumstances, STAT3 is activated in a transient manner with activation terminated by suppressors of cytokine signaling (SOCS) proteins. STAT3 is activated by phosphorylation at tyrosine 705 (Y705) and serine 727 (S727) [10]. Phosphorylation of STAT3 at Y705 is often mediated by JAK1, epidermal growth factor receptor, or Src and is required for STAT3 homo- or hetero-dimerization, nuclear translocation and DNA binding. Several target genes of STAT3 have been identified, including proteins that are involved in cell survival and proliferation, such as Bcl-2, Bcl-xL, Mcl-1, Fas, cyclin D1, cyclin E1 and p21 [4,11,12]. Constitutively activated STAT3 is associated with IBD, where it modulates gut immune cell activation [13]; the *STAT3* gene is a susceptibility loci for IBD [14]. IL-6 is elevated in the serum and mucosa of patients with IBD, and the level of IL-6 in serum is an indicator of disease relapse [15]. Blocking IL-6 reduced disease severity in association with diminished STAT3 activation and lamina propria T-cell apoptosis [16]. Hence, with this evidence, IL-6/STAT3 pathway was selected as a key target to treat UC.

Gallic acid (3,4,5-trihydroxybenzoic acid, GA), is a type of phenolic acid that is found in various natural products, such as gallnuts, pineapples, sumac, oak bark, green tea, apple peels, tea leaves, grapes,

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Table 1
List of primers used in qRT-PCR.

No	Gene	Primer sequence	Primer length
1	TNF-α	Forward-5′-TGGTGACCAGGCTGTCGCTACA-3′	20
		Reverse-5′-TACAGTCACGGCTCCCGTGGG-3′	20
2	IL-1β	Forward-5′-TAGACAACTGCACTACAGGCTCCGA-3′	25
		Reverse-5′- GGGTCCGACAGCACGAGGCT-3′	20
3	IFNγ	Forward-5′-AACCCACAGGTCCAGCGCCAA-3′	21
		Reverse-5′-GCAACAGCTGGTGGACCACTCG-3′	22
4	IL-6	Forward-5′-ATGCTGGTGACAACCACGGCC-3′	21
		Reverse-5′-CCTCTGTGAAGTCTCCTCTCCGGAC-3′	25
5	IL-17	Forward-5′-CGTGGCCTCGATTGTCCGCC-3	20
		Reverse-5′-GGTTTCTTAGGGGTCAGCCGCG-3′	22
6	iNOS	Forward-5′-GCCAGGGTCACAACTTTACA-3	20
		Reverse-5′-TTCACCAAGGTGGCTGAGAAG-3′	21
7	COX-2	Forward-5′-TATGCCACCATCTGGCTTCG-3	20
		Reverse-5′-GTTGCTCATCACCCCACTCA-3′	20
8	β-Actin	Forward-5′-GGCGGACTGTTACTGAGCTG-3′	20
		Reverse-5′-CTGCGCAAGTTAGGTTTTGTCA-3′	22

strawberries, bananas, and lemons and also in red and white wine [17]. GA possesses many beneficial effects including anti-oxidant [18], anti-inflammatory [19], anti-diabetic [20] and anti-carcinogenic [21] properties. Recently Ma et al. [22] reported that pretreatment with GA attenuated Dimethylnitrosamine-induced acute liver injury, which might be attributed to its capability of inducing nrf2 translocation and subsequent expression of hemoxygenase-1 and Glutathione-S-Transferase-α. Previous reports have shown that GA downregulates matrix metalloproteinase-2 (MMP-2)/MMP-9 in human leukemia K562 cells [23] and inhibits gastric cancer cell metastasis and invasive growth via increased expression of RhoB, downregulation of AKT/small GTPase signals and inhibition of NF-κB activity [21]. GA shows selective cytotoxicity for cancer cells and has little toxicity against normal cells [24]. In the present study, we analyzed the anti-colitic effect of gallic acid in BALB/c mice with DSS-induced colitis. We found that GA potentially suppressed pro-inflammatory cytokines (TNF-α, IL-1β, IL-17 and IFN-γ) and inflammatory mediators, such as iNOS and COX-2. Furthermore, GA treatment inhibited the activation and translocation of p65-NF-κB and IL-6/STAT3 pathways.

2. Materials and methods

2.1. Chemicals

HistoVT antigen retrieval kit (10×, pH 7.0), propidium iodide, the BCA protein assay kit and the peroxidase stain DAB kit were purchased from Nacalai Tesque (Kyoto, Japan). Primary antibodies against iNOS, COX-2, Bcl-xL and actin were procured from Santa Cruz Biotechnology (Dallas, TX, USA); antibodies against p-STAT3^{Y705} and T-STAT3 were from Cell Signaling Technology (Danvers, MA, USA). QIAshredder and RNeasy Kit and QuantiFast SYBR Green PCR Master Mix were purchased from QIAGEN (Hilden, Germany), and Clarity western ECL substrate and Immun-Blot® PVDF membrane were from BIO-RAD (USA). All other chemicals used were analytical grade.

2.2. Animals

Male BALB/c mice weighing 25–30 g were purchased from A Sapphire Enterprises, Seri Kembangan, Selangor, Malaysia. The mice were housed individually in plastic cages at a constant temperature (21 ± 2 °C) with an alternating 12-h light/dark cycle; animal chow and water were provided *ad libitum*. All animal treatments adhered strictly to institutional and international ethical guidelines of the care and use of laboratory animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia for the use of animal subjects.

2.3. Experimental design

Animals were randomly divided in 3 experimental groups. Group 1 served as control received normal drinking water. In Group 2, acute

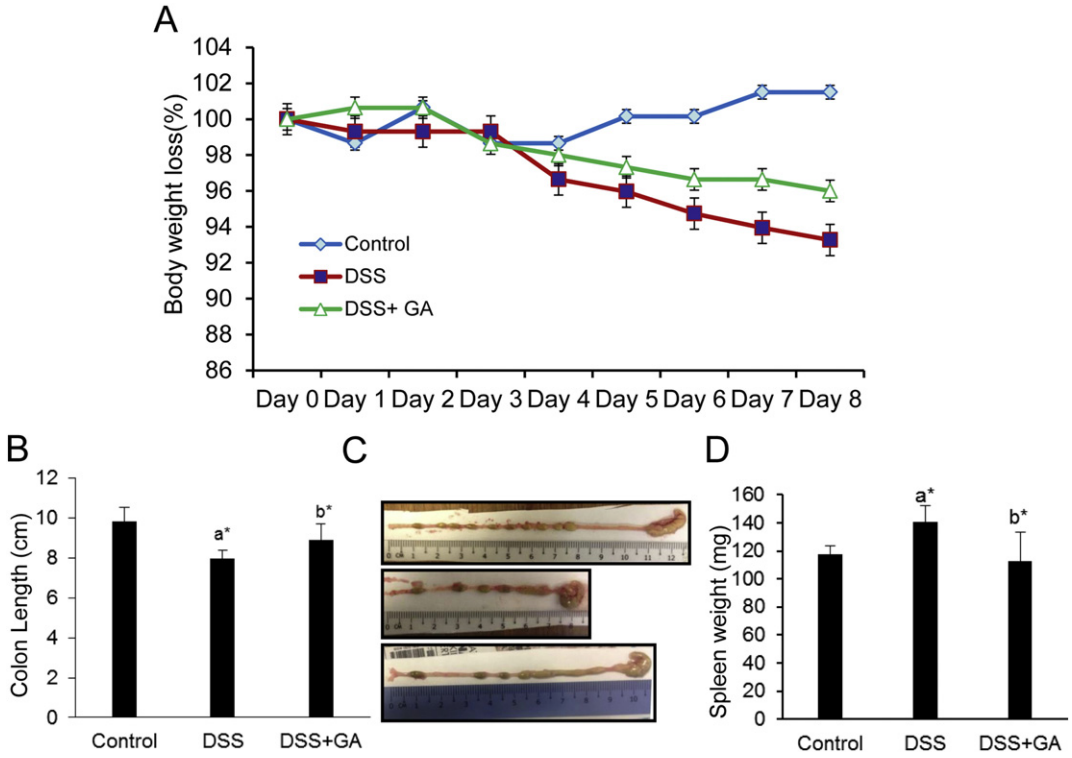

Fig. 1. (A) The effect of gallic acid on body weight loss, (B and C) colon length, and (D) spleen weight. The values are expressed as the mean ± S.D. Comparisons: [a]Control vs. DSS, [b]DSS vs. DSS + GA. *, significant at $p < 0.05$; ns, non-significant.