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Quercus infectoria inhibits Set7/NF-κB inflammatory pathway in macrophages exposed to a diabetic environment

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ABSTRACT

Chronic inflammation plays a key role in the pathogenesis of myriad complications associated with diabetes and thus anti-inflammatory therapies may ameliorate these complications. *Quercus infectoria* (Qi) extract has been shown to downregulate inflammatory processes; however, the molecular mechanisms of this anti-inflammatory activity remain unclear. The hypothesis of our study was that Qi extract exerts its anti-inflammatory effect by downregulating the Set7/NF-κB pathway. Bone marrow-derived macrophages (BMM) were treated with high glucose plus palmitate medium (HG/Pa) to simulate the diabetic environment. Compared with control conditions, HG/Pa elevated expression Set7, expression and activity of NF-κB along with expression of several inflammatory cytokines. These changes were associated with increased levels of intracellular reactive oxygen species (ROS). Moreover, similar alterations were demonstrated in BMM derived from mice fed a high fat diet (HFD) compared to those from lean mice, suggesting that HFD-induced changes in BM progenitors persist throughout differentiation and culture. Importantly, Qi extract dose-dependently reduced Set7, p65 and inflammatory cytokine expression relative to vehicle controls in both HG/Pa-and HFD-treated BMM. Finally, macrophages/monocytes isolated from wounds of diabetic mice that were treated with Qi solution exhibited lower expression of the inflammatory cytokines, IL-1β and TNF-α, compared with vehicle treated wounds, demonstrating translation to the in vivo diabetic environment. Taken together, data from this study suggests that Qi downregulates diabetes-induced activity of the Set7/NF-kB pathway.

1. Introduction

Diabetes is a significant health problem world-wide and is closely associated with myriad complications. Hyperglycemia is a hallmark of diabetes and is thought to contribute to the development of neuropathy, cardiovascular diseases, atherosclerosis, and chronic wounds. Hyperglycemia may promote diabetic complications in part by promoting inflammation via several pathways, especially those involving the transcription factor NF-κB [1,2]. NF-kB can be considered a master regulator of inflammatory mediators and oxidative molecules [3,4]. Thus, therapies that inhibit hyperglycemia-induced NF-kB activity may ameliorate chronic inflammation and complications associated with diabetes

Nutgall of *Quercus infectoria* (Qi) G. Olivier (Fagaceae) has been used for centuries in traditional medicine in several Asian countries for the treatment of infectious diseases and inflammatory disorders [5]. Previous researchers have identified a number of pharmacological activities of Qi extract including antimicrobial [6], anti-inflammatory [7,8], and anti-oxidant activity [9]. Qi extract contains high concentrations of tannins such as gallic acid, ellagic acid, along with flavonoids that are known to have anti-inflammatory properties. Although many studies have attempted to identify the mechanism(s) by which Qi extract inhibits inflammation [7,8], the pathways involved in this anti-inflammatory activity remain unclear.

Set7 is an enzyme that methylates lysine residues of both histone and non-histone proteins, including NF-κB, and has been shown to contribute to hyperglycemia-induced inflammation in animal and cell culture models [10–12]. In humans, diabetes is associated with increased levels of Set7 in peripheral blood mononuclear cells, which in turn is associated with epigenetic modifications on the NF-κB p65 promotor and upregulation of NF-κB p65 along with target inflammatory cytokines [13]. Moreover, Set7 has been implicated in the regulation of anti-oxidant enzyme expression and the accumulation of intracellular reactive oxygen species (ROS) [14]. Thus, the hypothesis of our study was that Qi extract exerts its anti-inflammatory effect by inhibiting the Set7/NF-κB inflammatory pathway.

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Table 1
PCR primers.

	Forward	Reverse
GAPDH	TCTGACGTGCCGCCTGGAGA	GGGGTGGGTGGTCCAGGGTT
IL-1β	ATGCCACCTTTTGACAGTGATG	CAGGTCAAAGGTTTGGAAGCA
TNF-α	TTCCAGATTCTTCCCTGAGGT	TAAGCAAAAGAGGAGGCAACA
IL-6	GCTGGTGACAACCACGGCCT	GGCATAACGCACTAGGTTTGCCG
NF-κB p65	GGGCCGGGAACGGGA	GGCTGTTTGTCCCGAGGC
SET7	CCGTGGAAGGGCACCT	GGAGTAGGTGACAGTGCAGA

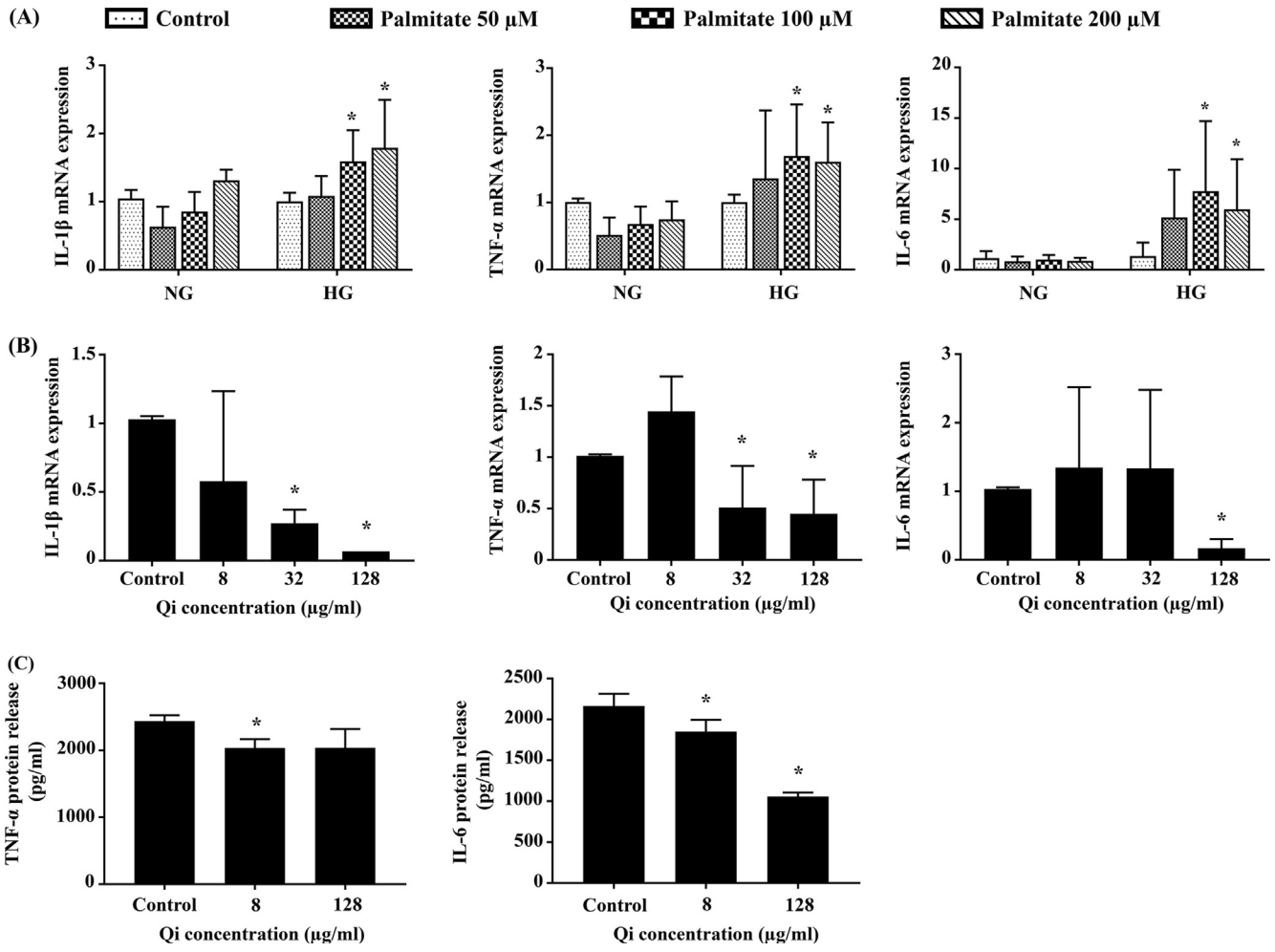

Fig. 1. High glucose/palmitate increases and Qi treatment reduces inflammatory cytokine expression in macrophages. (A) mRNA expression of inflammatory cytokines in bone marrow-derived macrophages (BMM) cultured with normal glucose (NG) or high glucose (HG) with/without palmitate at different concentrations for 24 h. (B) mRNA expression and (C) protein release of inflammatory cytokines in BMM cultured with HG plus palmitate at 200 μM with or without Qi extract at different concentrations or vehicle. All genes were normalized to GAPDH and then to vehicle control. Data shown as mean ± SE (n = 6). $^{*}p < 0.05$compared with control.

2. Materials and methods

2.1. Animals

Diabetic db/db mice and non-diabetic wild-type C57Bl/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). Transgenic mice expressing *Photinus* luciferase cDNA under HIV-LTR (HLL) were used for luciferase assay [15]. For high fat diet-induced (HFD) mice, male C57Bl/6 mice were fed a high fat diet (60 kcal% fat diet, D12492 from Research Diets) for 18 weeks to induce obesity and insulin resistance. Experiments with db/db, normal chow- and HFD-fed C57Bl/6, and HLL mice were performed on 12–16 week-old mice. All experimental procedures were approved by the Animal Care Committee at the University of Illinois at Chicago.

2.2. Preparation of Qi extract and Qi solution

Nutgalls of *Quercus infectoria* were obtained from Thai Herbal shop, Songkhla, Thailand. The dry plant materials were extracted with 95% ethanol in the ratio 1:10 for 7 days. The solution was filtered and evaporated to dryness. Then, the extract was dissolved in 10% dimethylsulfoxide to obtain as a stock solution at a concentration 100 mg/ml. For in vivo experiment, Qi solution at 30% (w/v) of concentration was pharmaceutically formulated as a topical wound treatment including propylene glycol, polyethylene glycol 400, polysorbate 20, polysorbate 60, 95% ethanol, and distilled water.

2.3. Cell culture and treatment

Bone marrow-derived macrophages (BMM) were cultured as de-