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Brazilian propolis-derived components inhibit TNF-α-mediated downregulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes

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ABSTRACT

Background: Previous reports suggest that Brazilian propolis has multiple biological functions and may help to restore adiponectin expression and insulin sensitivity. However, little is known about the molecular mechanisms by which these compounds inhibit the downregulation of adiponectin.
Methods: The effect of various Brazilian propolis-derived components on inhibition of tumor necrosis factor-α (TNF-α)-mediated downregulation of adiponectin expression in 3T3-L1 adipocytes and molecular mechanism was investigated.
Results and conclusions: Pretreatment with either artepillin C (C3) or its derivative (C4) significantly inhibited TNF-α-mediated downregulation of adiponectin expression in 3T3-L1 adipocytes. Interestingly, C3 strongly activated peroxisome proliferator-activated receptor γ (PPARγ) transcriptional activity. Treatment of adipocytes with C3 resulted in the upregulation of adiponectin and fatty acid-binding protein 4 expression, but C4 did not significantly induce PPARγ transactivation. C4 did, however, inhibit the TNF-α-induced c-Jun-NH_2-terminal kinase (JNK) signaling that is involved in adiponectin expression. Molecular docking studies based on hPPARγ with C3 and JNK1 with C4 clearly supported our experimental results. These data demonstrate that 1) both C3 and C4 significantly inhibit the TNF-α-mediated downregulation of adiponectin in adipocytes, 2) C3 functions as a PPARγ agonist, and its inhibition of the effect of TNF-α is due to this PPARγ transactivation, and 3) C4 is an effective inhibitor of JNK activation, thus inhibiting the TNF-α-mediated downregulation of adiponectin.
General significance: Brazilian propolis-derived components (C3 and C4) can significantly inhibit TNF-α-mediated downregulation of adiponectin in adipocytes, although they do so via different mechanisms.

1. Introduction

Adiponectin is specifically and highly expressed in adipocytes. Plasma adiponectin concentration and mRNA expression levels are decreased in the obese and insulin-resistant states [1,2]. Administration of adiponectin improves insulin action and is accompanied by an increase in fatty acid oxidation and a decrease in triacylglycerol level in muscle [3].

Obesity is associated with macrophage infiltration into the adipose tissue and the activation of the inflammatory pathway, which leads to the development of insulin resistance [4,5]. TNF-α secreted from infiltrated macrophages has a causal role in adipocyte dysfunction, including the downregulation of adiponectin expression [6–8]. Therefore, anti-inflammatory compounds may ameliorate the adipocyte dysfunction associated with metabolic syndrome by inhibiting the downregulation of adiponectin expression.

Therapeutic regimens are currently being considered which will target the regulation of adipocyte function, in order to improve insulin sensitivity or glucose homeostasis. The peroxisome proliferator-activated receptor γ (PPARγ), a ligand-dependent transcription factor of the nuclear hormone receptor superfamily, regulates the transcription of many genes involved in cellular lipid homeostasis, adipocyte differentiation, and insulin action [9]. Thiazolidinediones are among

Abbreviations: aP2, adipocyte fatty acid-binding protein 4; E_{MM}, molecular mechanics energy; FBS, fetal bovine serum; JNK, c-Jun-NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MKK, mitogen-activated protein kinase kinase; MM-PBSA, molecular mechanics Poisson-Boltzmann surface area; PPAR, peroxisome proliferator-activated receptor; SEK1/MKK4, stress-activated protein kinase/extracellular signal-regulated kinase kinase 1/mitogen-activated protein kinase kinase 4; TNF-α, tumor necrosis factor-α; TTBS, Tris–HCl-buffered saline containing 0.05% Tween 20; TZD, troglitazone; vdW, van der Waals

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the synthetic PPARγ ligands and are used clinically as anti-diabetic drugs [9].

Recently, considerable attention has been focused on food factors that may be beneficial in reducing the risk of metabolic syndrome. Although various drugs have been used therapeutically for obesity-related metabolic diseases, there has been little evidence that food factors can be directly beneficial or that they can mitigate the disease-induced dysfunction of adipocytes which are responsible for adipocytokine expression and insulin sensitivity.

Propolis is a resinous substance collected by honeybees from various plant sources. It is thought to be produced in beehives as a protective barrier against enemies. Its nutraceutical properties are well known in folk medicine. Propolis, which is now extensively used in food and beverages to improve health and prevent disease, contains a variety of chemical compounds such as polyphenols, terpenoids, steroids, and amino acids [10–14]. It has been reported to possess various biological activities due to its antibacterial [15], antiviral [16], anti-inflammatory [17], anticancer [18,19], and antifungal [20] properties. The composition of propolis depends on the place and time of collection. Artepillin C and other prennyl cinammic acid derivatives have been identified in Brazilian propolis [21,22] and Kumazawa et al. demonstrated that the botanical origin of Brazilian propolis is *Baccharis dracunculifolia*[23] (Fig. 1). These compounds have potent neuroprotective [24] and antiangiogenic effects [25] as well as inhibit the release of cys-leukotrienes [26].

These previous reports suggest that Brazilian propolis has multiple biological functions and may help to restore adiponectin expression and insulin sensitivity. However, little is known about the molecular mechanisms by which these compounds inhibit the downregulation of adiponectin. In the present study, we demonstrate that (*E*)-3-(4-hydroxy-3,5-bis(3-methylbut-2-enyl)phenyl)acrylic acid (artepillin C; C3) and (*E*)-3-(4-(isobutyryloxy)-3-(3-methylbut-2-enyl)phenyl) acrylic acid (C4) can significantly inhibit the downregulation of adiponectin expression that is induced by TNF-α in 3T3-L1 adipocytes. The present study demonstrates that both C3 and C4 function by inhibiting this downregulation of adiponectin, although they do so via different molecular mechanisms.

2. Materials and methods

2.1. Chemicals

p-coummaric acid (C1) and artepillin C (C3) were purchased from Wako Pure Chemical (Osaka, Japan). (*E*)-3-(4-hydroxy-3-(3-methylbut-2-enyl) phenyl)acrylic acid (drupanin; C2), (*E*)-3-(4-(isobutyryloxy)-3-(3-methylbut-2-enyl)phenyl)acrylic acid (C4), (*E*)-3-(3-(3-methylbut-2-enyl)-4-(3-phenylpropanoyloxy)phenyl)acrylic acid (baccharin; C5), and (*E*)-3-(3-hydroxy-2,2-dimethyl-8-(3-methylbut-2-enyl)chroman-6-yl)acrylic acid (C6) were extracted from Brazilian propolis using 75% ethanol and purified using preparative HPLC [21,22]. Purity of all isolated compounds was more than 95%. Troglitazone (TZD) and GW9662 were purchased from Cayman Chemical (Ann Arbor, MI). GW7647 was purchased from Sigma-Aldrich (St. Louis, MO). Murine TNF-α (recombinant) was purchased from R&D Systems (Minneapolis, MN). Anti-c-Jun-NH_2-terminal kinase (JNK), anti-phospho-JNK (Thr183/Tyr185), anti-stress-activated protein kinase/extracellular signal-regulated

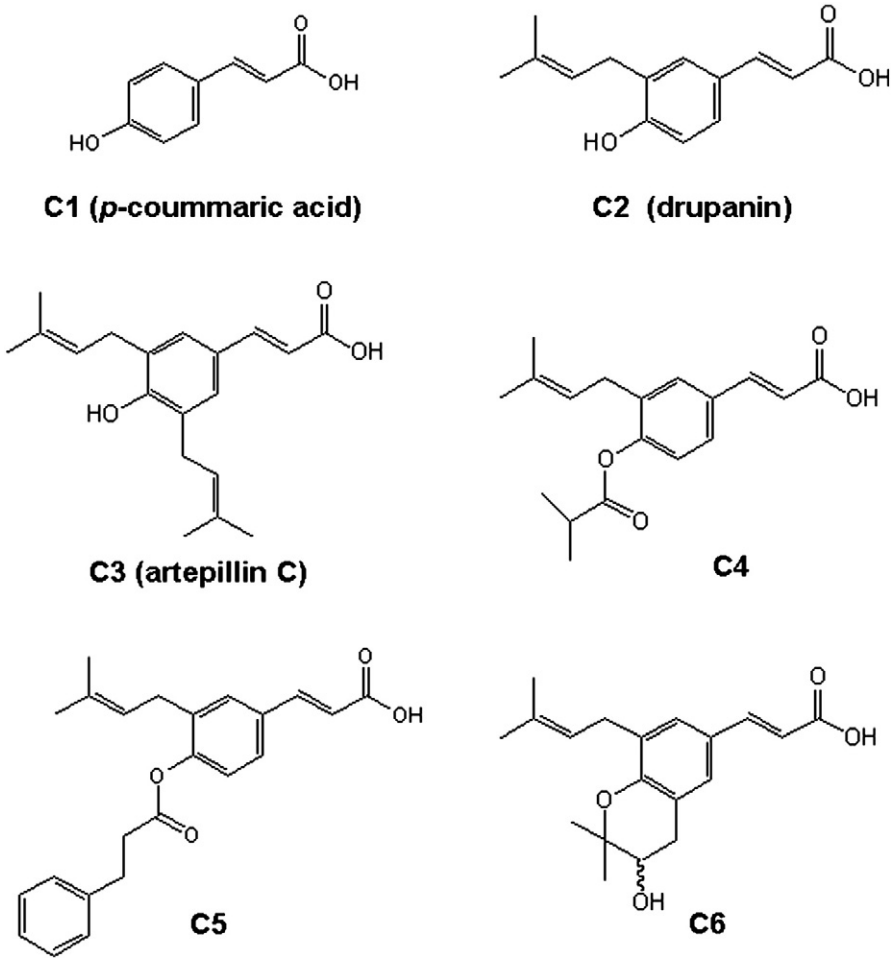

Fig. 1. Chemical structure of the propolis-derived components.