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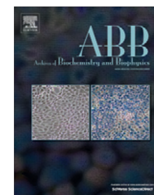

(−)-Epicatechin prevents TNFα-induced activation of signaling cascades involved in inflammation and insulin sensitivity in 3T3-L1 adipocytes

Marcela A. Vazquez-Prieto [a,b], Ahmed Bettaieb [c], Fawaz G. Haj [c], César G. Fraga [c,d], Patricia I. Oteiza [c,e,*]

[a] Department of Pathology, School of Medicine, National University of Cuyo, Argentina
[b] Laboratory of Cardiovascular Pathophysiology, Institute for Experimental Medical and Biological Research (IMBECU)-CONICET, Mendoza, Argentina
[c] Department of Nutrition, University of California, Davis, USA
[d] Physical Chemistry-IBIMOL (UBA-CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires-CONICET, Argentina
[e] Department of Environmental Toxicology, University of California, Davis, USA

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ABSTRACT

Obesity is major public health concern worldwide and obese individuals exhibit a higher risk of chronic diseases such as type 2 diabetes. Inflammation plays a significant role in metabolic regulation and mounting evidence highlight the contribution of adipose tissue to systemic inflammatory state. Food extracts with a high content of (−)-epicatechin have been found to exert systemic anti-inflammatory actions, however the anti-inflammatory actions of (−)-epicatechin on adipose tissue remain to be determined. The aim of this study was to investigate the capacity of (−)-epicatechin to prevent tumor necrosis alpha (TNFα)-induced activation of cell signals involved in inflammation and insulin resistance (NF-κB, mitogen-activated protein kinases (MAPKs), AP-1, and peroxisome proliferator activated receptor γ (PPARγ)) in differentiated white adipocytes (3T3-L1). TNFα triggered the activation of transcription factors NF-κB and AP-1, and MAPKs ERK1/2, JNK, and p38. (−)-Epicatechin caused a dose (0.5–10 μM)-dependent decrease in TNFα-mediated JNK, ERK1/2, and p-38 phosphorylation, and nuclear AP-1-DNA binding. (−)-Epicatechin also inhibited TNFα-triggered activation of the NF-κB signaling cascade, preventing TNFα-mediated p65 nuclear transport and nuclear NF-κB-DNA binding. (−)-Epicatechin also attenuated the TNFα-mediated downregulation of PPARγ expression and decreased nuclear DNA binding. Accordingly, (−)-epicatechin inhibited TNFα-mediated altered transcription of genes (MCP-1, interleukin-6, TNFα, resistin, and protein-tyrosine phosphatase 1B) involved in inflammation and insulin signaling. In conclusion, (−)-epicatechin can attenuate TNFα-mediated triggering of signaling cascades involved in inflammation and insulin resistance. These findings could be of relevance in the dietary management of obesity and metabolic syndrome.

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Introduction

Obesity and metabolic syndrome result from excess calorie intake and genetic predisposition, and obese individuals exhibit a higher risk of chronic diseases such as type 2 diabetes [1–3]. Mounting evidence highlight the contribution of adipose tissue to a systemic inflammatory state, which can play a significant role in metabolic regulation [4,5]. Adiposity promotes the secretion of proinflammatory cytokines, such as tumor necrosis factor alpha (TNFα), interleukin (IL)-6, resistin, monocyte chemoattractant protein-1 (MCP-1), by both adipocytes and infiltrated inflammatory cells, including recruited macrophages. These proinflammatory cytokines contribute to the sustained adipose and systemic inflammation and insulin resistance associated with obesity [6]. A significant role of TNFα on these events was initially suggested by the findings that adipose-secreted TNFα contributed to insulin resistance in rodent models of obesity [7], and that obese mice lacking a functional TNFα are protected from dietary obesity-induced insulin resistance [8].

TNFα is a major player mediating the activation of signaling cascades in adipocytes that are central to inflammation and insulin resistance. In this regard, TNFα triggers adipocyte activation of the mitogen activated kinases (MAPKs) extracellular-signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK), and p38, and of

Abbreviations used: DIFM, differentiation medium; EMSA, electrophoretic mobility shift assay; ERK, extracellular-signal-regulated kinases; IL, interleukin; IR, insulin receptor; IRS-1, insulin receptor subtrates-1; JNK, c-Jun N-terminal kinases; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MCP-1, monocyte chemoattractant protein 1; NF-κB, nuclear factor kappa B; PPARγ, peroxisome proliferator-activator receptor gamma; PTP1B, protein-tyrosine phosphatase 1B; TNFα, tumor necrosis factor alpha.

* Corresponding author. Address: Department of Nutrition, University of California, Davis, One Shields Avenue, Davis, CA 95616. Fax: +1 530 752 8966.
E-mail address: poteiza@ucdavis.edu (P.I. Oteiza).

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transcription factors AP-1 and nuclear factor kappa B (NF-κB) [9–11]. These signaling cascades are in part redox regulated, given that binding of TNFα to its receptor results in the activation of NADPH oxidase leading to an increase in oxidant production [12,13]. Activation of these signaling pathways leads to an increased expression of pro-inflammatory cytokines, including IL-6, IL-8, IL-1β, and MCP-1. NF-κB also drives [14] an increased expression of protein-tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signaling, which dephosphorylates tyrosine residues of the insulin receptor (IR) and insulin receptor substrate1 (IRS1) [15]. Furthermore, TNFα down regulates the nuclear receptor transcription factor peroxisome proliferator-activator receptor gamma (PPARγ) that plays a major role in the regulation of both adipose glucose and lipid metabolism [14].

Flavonoids are polyphenolic compounds that are widely present in human diets. Epidemiological studies have shown an inverse relationship between consumption of flavonoid-rich foods and pathologies with inflammatory components [16]. The beneficial health effects of flavonoids can be in part attributed to their capacity to regulate oxidant production and pro-inflammatory signals [17]. Recent reports support an anti-inflammatory action of select polyphenols in adipocytes in cultures. Addition of a polyphenol-rich grape powder extract to human adipocytes, inhibits TNFα-triggered activation of MAPKs and NF-κB, and the expression of proteins involved in inflammation and insulin resistance [18]. Similarly, high concentrations of the phenolic compounds p-coumaric acid, quercetin and resveratrol prevent TNFα-induced increase in several parameters of inflammation and oxidative stress, and of decreased insulin sensitivity in 3T3-L1 [19] and human adipocytes [9]. Oligomerized grape seed polyphenols attenuated inflammatory events in co-cultures of adipocytes and macrophages, through mechanisms attributed to their antioxidative properties [20].

(−)-Epicatechin is one of the most abundant flavonoids in human diets, being present in high concentrations in grapes, cocoa, tea, and many other fruits and vegetables. (−)-Epicatechin has a basic chemical structure of two aromatic rings (A and B) linked by an oxygenated heterocycle (C) with a hydroxyl group in position 4. Consumption or supplementation with (−)-epicatechin or (−)-epicatechin-containing foods in humans and experimental animals are associated with the improvement of parameters related to cardiovascular disease, which pathology involves a significant proinflammatory component [21,22].

In vitro, (−)-epicatechin modulates the production of reactive oxygen species, activation of NF-κB, and production of cytokines in Jurkat T cells and Hodgkin's lymphoma cells [23,24]. However, the capacity of (−)-epicatechin to attenuate inflammation in adipocytes has not been yet characterized. This study investigated in 3T3-L1 adipocytes, the capacity of (−)-epicatechin to inhibit TNFα-triggered deregulation of signaling pathways (MAPKs ERK1/2, JNK and p38, and transcription factors NF-κB, AP-1, and PPARγ) that regulate genes involved in inflammation and insulin resistance.

Materials and methods

Materials

3T3-L1 and Raw 264.7 cells were obtained from the American Type Culture Collection (Rockville, MA, USA). Cell culture media and reagents, and TRIzol reagent were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). Antibodies for ERK (sc-93), p-Erk (sc-7383), heterogeneous nuclear ribonucleoprotein A1 (sc-32301), JNK (sc-572), p-JNK (sc-6254), PPARγ (7273), MCP-1 (1785), TNFα (sc-1351); and the oligonucleotide containing the consensus sequence for PPAR were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody for p-p38 (9211) was obtained from Cell Signaling Technology (Danvers, MA, USA). The antibody for PTP1B was from Abcam Inc. (Cambridge, MA). PVDF membranes were obtained from BIO-RAD (Hercules, CA, USA) and Chroma Spin-10 columns were from Clontech (Palo Alto, CA, USA). The ECL Western blotting system was from GE Healthcare (Piscataway, NJ, USA). The oligonucleotides containing the consensus sequence for NF-κB, and AP-1, the reagents for the electrophoretic mobility shift assays (EMSA), and cDNA reverse transcriptase were obtained from Promega (Madison, WI, USA). (−)-Epicatechin, TNFα, lipopolysaccharide (LPS), and all other reagents were from the highest quality available and were purchased from Sigma (St. Louis, MO, USA).

Cell culture and incubations

3T3-L1 preadipocytes were maintained in DMEM containing 25 mM glucose, 10% (v/v) new born calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. To induce cell differentiation, 3T3-L1 pre-adipocytes were grown to confluence in culture medium containing 10% (v/v) fetal bovine serum. Confluent cells were then switched to differentiation medium (DIFM) containing 20% (v/v) fetal bovine serum, 20 nM insulin and 1 nM triiodothyronine for 48 h. Adipocyte differentiation was induced by treating cells for 48 h in DIFM further supplemented with 0.5 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 0.125 mM indomethacin (induction media). After induction, cells were returned to DIFM medium, exhibiting at day 12 a fully differentiated phenotype with massive accumulation of multilocular and unilocular fat droplets. 3T3-L1 adipocytes were treated with 0.5–10 μM (−)-epicatechin during 4 h, and subsequently treated with TNFα (20 ng/ml) for 15 min-24 h depending on the determination.

RAW 264.7 macrophages were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum. Cells were preincubated for 4 h in the absence or the presence of 1 and 10 μM (−)-epicatechin, and subsequently for 24 h without or with 0.5 μg/ml lipopolysaccharide (LPS). TNFα concentration in the cell culture media was determined by ELISA using a commercial kit (BD Biosciences, San Diego, CA) according to the manufacturer's protocol.

Western blot analysis

To prepare total extracts, cells were rinsed with PBS, scraped and centrifuged. The pellet was rinsed with PBS, and resuspended in 200 μL of 50 mM HEPES (pH 7.4), 125 mM KCl contained containing protease inhibitors and 2% (v/v) Igepal. The final concentration of the inhibitors was 0.5 mmol/L PMSF, 1 mg/L leupeptin, 1 mg/L pepstatin, 1.5 mg/L aprotinin, 2 mg/L pestatin and 0.4 mM sodium pervanadate. Samples were exposed to one cycle of freezing and thawing, incubated at 4 °C for 30 min and centrifuged at 15,000*g* for 30 min. The supernatant was decanted and protein concentration was measured (Bradford, 1976). Aliquots of total cell lysates containing 25–40 μg protein were denatured with Laemmli buffer, separated by reducing 10–12.5% (w/v) polyacrylamide gel electrophoresis, and electroblotted to PVDF membranes. Membranes were blotted for 2 h in 5% (w/v) non-fat milk, and subsequently incubated in the presence of corresponding primary antibodies (1:1000 dilution for all the antibodies except for TNFα (1:500) and PTP-1B (1:5000)) overnight at 4 °C. After incubation for 90 min at room temperature in the presence of the secondary antibody (HRP conjugated) (1:10,000 dilution) the conjugates were visualized and quantified by chemiluminescence detection in a Phosphoimager 840 (Amersham Pharmacia Biotech. Inc., Piscataway, NJ).

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