Curcumin, a Potential Inhibitor of Up-regulation of TNF-alpha and IL-6 Induced by Palmitate in 3T3-L1 Adipocytes through NF-kappaB and JNK Pathway[1]

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Objective To investigate the attenuating effect of curcumin, an anti-inflammatory compound derived from dietary spice turmeric (*Curcuma longa*) on the pro-inflammatory insulin-resistant state in 3T3-L1 adipocytes. **Methods** Glucose uptake rate was determined with the [^{3}H] 2-deoxyglucose uptake method. Expressions of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by quantitative RT-PCR analysis and ELISA. Nuclear transcription factor kappaB p65 (NF-κB p65) and mitogen-activated protein kinase (MAPKs) were detected by Western blot assay. **Results** The basal glucose uptake was not altered, and curcumin increased the insulin-stimulated glucose uptake in 3T3-L1 cells. Curcumin suppressed the transcription and secretion of TNF-α and IL-6 induced by palmitate in a concentration-dependent manner. Palmitate induced nuclear translocation of NF-kB. The activities of Jun NH2-terminal kinase (JNK), extracellular signal–regulated kinase1/2 (ERK1/2) and p38MAPK decreased in the presence of curcumin. Moreover, pretreatment with SP600125 (inhibitor of JNK) instead of PD98059 or SB203580 (inhibitor of ERK1/2 or p38MAPK, respectively) decreased the up-regulation of TNF-α induced by palmitate. **Conclusion** Curcumin reverses palmitate-induced insulin resistance state in 3T3-L1 adipocytes through the NF-kB and JNK pathway.

Key words: Curcumin; Insulin resistance; Inflammation; Adipocyte; Free fatty acids

INTRODUCTION

Although type 2 diabetes is closely associated with obesity, the mechanisms by which obesity leads to type 2 diabetes remain unclear. Insulin resistance is a common pathogenesis of obesity and type 2 diabetes because obesity leads to hyperlipidemia. High level of free fatty acids (FFAs) in plasma and tissue reduces insulin sensitivity, impairs insulin signaling and induces insulin resistance[1–8].

Pro-inflammatory cytokines play a critical role in the development of insulin resistance[9] and their levels are elevated in insulin-resistant states like obesity and type 2 diabetes[10-13]. *In vivo* and *in vitro* studies demonstrated that activation of pro-inflammatory pathways is mechanically linked to insulin resistance, and that the NF-κB pathway plays a critical role in lipid-induced insulin resistance. Moreover, data suggest that FFA-derived metabolic products can activate JNK, NF-κB, and protein kinase θ (PKCθ)[14-17], all of which can phosphorylate insulin receptor substrate-1 (IRS-1) on serine residues. Consequently, IRS-1 activation through tyrosine phosphorylation is impaired, leading to a reduction in insulin receptor-mediated signaling and subsequent insulin resistance.

Curcumin derived from the rhizome of the herb *Curcuma longa* has been used for centuries in Asia as a dietary spice, and may be of therapeutic benefits to several diseases[18-21]. Curcumin has been used traditionally as an antidiabetic agent[22-24]. Its potential antidiabetic effect is determined, based on murine animal models. Previous studies have confirmed that oral curcumin treatment improves hyperglycemia in KK-Ay mice and streptozotocin-treated rats[25-29]. Curcumin also exerts potential anti-inflammatory effects by inhibiting pro-inflammatory cytokines and chemokines, adhesion molecules, cyclooxygenase-2, tissue factor and inducible nitric oxide synthase in diverse cell types (pancreatic cells, chodrocytes, and

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hepatic cells)[30-31]. These suppressive effects are due to the inhibition of the NF-kB pathway and other pro-inflammatory signaling pathways including MAPKs[33-36]. Although these pro-inflammatory signaling pathways might be involved in the pathogenesis of type 2 diabetes, there is no evidence that curcumin is an anti-inflammatory agent against obesity-induced insulin resistance. The present study was designed to study the underlying mechanism of curcumin to reduce pro-inflammatory cytokines in 3T3-L1 adipocytes with FFA- induced insulin resistance. Our specific aim was to investigate whether the effect of curcumin on adipokins is dependent on the NF-κB and MAPKs pathways. The results of this study may provide potential evidence for the treatment of obesity and type 2 diabetes with curcumin.

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle's medium and fetal bovine serum (FBS) were purchased from GIBCO (BRL, USA). Curcumin, palmitate, SP600125, PD98059 and SB203580 were purchased from Sigma-Aldrich (St. Louis, MO). [^{3}H] 2-deoxyglucose was obtained from PerkinElmer Life and Analytical Sciences. Rabbit antibody to SAPK/JNK (Thr183/ Thr185), phospho-SAPK/JNK (Thr183/Thr185), p38MAPK, phospho-p38MAPK, p44/ 42MAPK (Thr202/Tyr204), phospho-p44/42MAPK (Thr202/ Tyr204) were purchased from Cell Signaling (Beverly, MA). NF-κB p65 antibody and HRP-conjugated anti-rabbit antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ECL plus Western blot detection system was purchased from Amersham Biosciences (GE Healthcare, UK). TNF-α, IL-6 ELISA kit were purchased from Jingmei (Jingmei Biotech, China).

Cell Culture and Treatment

3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA) were grown in DMEM containing 10% fetal bovine serum (FBS) and fed every 2 days. Two days after confluence (day 0), the medium was switched to DMEM supplemented with 10% FBS, 5 μg/mL insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine and 1 μmol/L dexamethasone. On day 2, the medium was changed to DMEM containing 10% FBS and 5 μg/mL insulin. Beginning on day 4, the medium was changed to DMEM containing only 10% FBS, and cells were given fresh medium every 2 days. Unless indicated otherwise, adipocytes were used 10-12 days after differentiation. After DMEM supplemented with 0.5% FFA-free bovine albumin, 3T3-L1 adipocytes were treated with 0.25 mmol/L palmitate in the presence of 0.5% FFA-free BSA with or without the indicated doses of curcumin (final concentration: 5 μmol/L,10 μmol/L, 20 μmol/L) for 24 h.

[^{3}H] 2-Deoxyglucose Uptake Assay

3T3-L1 preadipocytes (5×10^5/well) were differentiated to adipocytes in a 24-well plate. After serum-starvation in 0.2% BSA DMEM overnight, the cells were incubated in 0.2% BSA DMEM containing 0.25 mmol/L PA or (and) 5 μmol/L, 10 μmol/ L or 20 μmol /L curcumin for 24 or 48 h. The cells were then incubated in 1 mL Krebs/ Ringer phosphate (KRP)/HEPES (131.2 mmol/L NaCl, 4.71 mmol/L KCl, 2.47 mmol/L $CaCl_2$, 1.24 mmol/L $MgSO_4$, 2.48 mmol/L Na_3PO_4, 10 mmol/L HEPES, pH 7.4) with or without 100 nmol/L insulin for 30 min at 37 ℃, after washed three times in KRP/HEPES buffer. Finally the cells were incubated in 1 mL KRP/HEPES containing 0.5 μCi/mL 2-deoxy-D-[^{3}H] glucose for 10 min at 37 ℃. The cells were washed three times with ice-cold PBS and solubilized in 1 mL 0.1 mol/L NaOH for 2 h. Radioactivity was determined by liquid scintillation spectrometry. Non-specific deoxyglucose uptake was measured in the presence of 20 μmol/L cytochalasin B, and specific glucose uptake was detected from the subtracted total uptake. Three replicate wells were set up and each experiment was performed in triplicate.

Cell Lysates and Western Blot

Cells were washed with phosphate-buffered saline and lysis buffer [1% Triton X-100, 50 mmol/L KCl, 25 mmol/L HEPES, pH 7.8, 10 μg/mL leupeptin, 20 μg/mL aprotinin, 125 μmol/L dithiothreitol (DTT), 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L sodium orthovanadate] and then added to the cells. The lysate was centrifuged at 12 000 rpm for 10 min. Cytosolic and nuclear fractions were separated (first buffer: 10 mmol/L HEPES pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EDTA, 1.5 mmol/L $MgCl_2$, 0.1% NP40, 1 mmol/L DTT; second buffer: 20 mmol/L HEPES pH 7.9, 420 mmol/L NaCl, 0.1 mmol/L EDTA, 1.5 mmol/L $MgCl_2$, 25% glycerol, 1 mmol/L DTT, 0.5 mmol/L PMSF). Protein concentrations were measured with a BCA protein assay kit (Pierce, USA). The lysate was boiled in a SDS loading buffer and applied on SDS-PAGE. Following gel transference, polyvinylidene difluoride (PVDF) membranes were blocked with 1% BSA in phosphate-buffered saline-Tween 20 for 1 h. Membranes were probed