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Effect of PPARα activation of macrophages on the secretion of inflammatory cytokines in cultured adipocytes

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Abstract

The relationship between adipocytes and infiltrated macrophages in fat tissue is important for the pathogenesis of insulin resistance through the activation of cytokines. Peroxisome proliferator-activated receptors (PPARs) play a role in the regulation of cytokine secretion in these cells. We studied the effect of the PPARα activation of macrophages on the modulation of the tumor necrosis factor α (TNFα) expression in adipocytes using a cell culture system. A conditioned medium of lipopolysaccharide (LPS)-stimulated RAW264.7 cells, a macrophage cell line, induced the level of TNFα mRNA in 3T3-L1 adipocytes. This effect was inhibited by the addition of neutralizing antibody against interleukin 6 (IL-6) in the conditioned medium or the preincubation of RAW264.7 cells with a specific PPARα agonist, K-111 (2,2-dichloro-12-(4-chlorophenyl)dodecanoic acid). K-111 reduced both the IL-6 production and mRNA expression in RAW264.7 cells, and its effect was stronger than that of rosiglitazone, a PPARγ agonist. The activation of the stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK) pathway and nuclear factor kappa B (NF-κB) subunits of p65 was significantly inhibited by K-111. The blocking of IL-6 production through the SAPK/JNK pathway or by transfection with siRNA specific for IL-6 abolished the inhibitory effect of K-111 on the TNFα expression in the 3T3-L1 adipocytes. As a result, the IL-6 produced by RAW264.7 cells is an inducer of TNFα expression in 3T3-L1 adipocytes, and the IL-6 secretion is inhibited by the activation of PPARα. The PPARα activators may suppress the pathogenetical secretion of TNFα in the adipocytes through the functional modulation of the infiltrated macrophages.

Keywords: Adipocyte; PPARα; Macrophage; Fat tissue; IL-6; TNFα

1. Introduction

Insulin resistance is linked to a wide array of metabolic disorders leading to atherosclerosis, such as hypertension, dyslipidemia, or disturbed glucose tolerance (Ginsberg, 2000; Hayden and Reaven, 2000; Reaven, 1995). A cluster of these abnormalities is now recognized as metabolic syndrome (Report of a WHO Consultation, 1999; Expert Panel on Detection, 2001). We have previously shown that visceral accumulation of adipose tissue, and not subcutaneous accumulation, causes systemic insulin resistance through the increased tumor necrosis factor α (TNFα) secretion from adipocytes using a cell-transplanted model (Shibasaki et al., 2002). Resistin is also another possible molecule, which regulates the systemic insulin sensitivity, possibly through the TNFα activation in the model mice (Kitagawa et al., 2004). In this context, the accumulated visceral fat secretes other cytokines, such as Vascular Endothelial Growth Factor (VEGF) (Miyazawa-Hoshimoto et al., 2005). As a result, the cytokine secretion of adipocytes accumulated in the visceral area seems to play an important role in the pathogenesis of insulin resistance and the related vascular diseases in humans. Recent transcriptional profiling experiments using animal models have pointed to a striking regulation of the inflammatory cytokines in adipose tissue, thus suggesting that macrophage infiltration into adipose tissue could be integral to these pathogenic changes (Weisberg et al., 2003; Xu et al., 2003). Interleukin 6 (IL-6) is one of the inflammatory cytokines which link both adipocytes and macrophages. The expression of IL-6 in fat tissue is elevated in individuals demonstrating

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obesity with insulin resistance (Mohamed-Ali et al., 1998, 1997; Vozarova et al., 2001; Straub et al., 2000; Fernandez-Real et al., 2001).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, which belong to the nuclear receptor family. PPARγ plays a pivotal function in the differentiation of adipocytes (Adams et al., 1997; Rosen and Spiegelman, 2001). In fact, chronic treatment with PPARγ activators improves the degree of glucose homeostasis by increasing the insulin sensitivity in various animal models of obesity and diabetes as well as in humans (Miyazaki et al., 2001; Hirose et al., 2002). However, a previous study showed that PPARγ agonists do not obviously suppress the IL-6 production in macrophage (Thieringer et al., 2000). PPARα was first identified for its role in the regulation of both the lipid and carbohydrate metabolisms, and subsequent data have also demonstrated that it exhibits a potent anti-inflammatory activity (Sheu et al., 2002; Delerive et al., 1999). Therefore, in this study we analyzed the effect of a PPARα agonist on the inflammatory cytokine expressions in adipocytes through the macrophage-derived IL-6 pathway using cultured cells, in order to elucidate the possible involvement of PPARα activation in the pathogenetical link between adipocytes and infiltrated macrophages in fat tissue.

2. Materials and methods

2.1. Materials

K-111 (2,2-dichloro-12-(4-chlorophenyl)dodecanoic acid, purity 99%), was synthesized at the Research Laboratories of Kowa company (Tokyo, Japan). Rosiglitazone was given from Takeda Pharmaceutical company (Osaka, Japan). SP600125 (Anthra[1,9-cd]pyrazol-6(2*H*)-one, purity 98%) was purchased from BIOMOL international L.P. (Plymouth Meeting, PA, USA) and these samples were used as solution of various concentrations in dimethyl sulfoxide (DMSO) purchased from SIGMA-Aldrich Company Ltd. (St. Louis, MO, USA). The final concentrations of DMSO were less than 0.1% where the cell viability was not affected. RAW264.7 cells and 3T3-L1 cells were obtained from ATCC (Dainippon Pharmaceutical, Osaka, Japan). DMEM containing 10 mM glucose (DMEM-L) medium and DMEM containing 25 mM glucose (DMEM-H) medium were obtained from SIGMA-Chemicals (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Gemini-Bio Products (Woodland, CA, USA). Lipopolysaccharide (LPS: *Escherichia coli* 0127 B8) was purchased from SIGMA-Chemicals (St. Louis, MO, USA). IL-6 Enzyme-Linked Immunosorbent Assay (ELISA) kits and Anti-mouse IL-6 neutralizing antibody were obtained from R&D systems (Minneapolis, MN, USA).

2.2. Cell culture

RAW264.7 cells were maintained in DMEM-L medium supplemented with 10% FBS, and gentamicin sulfate (20 mg/ml) at 37 °C under humidified 5% CO_2/95% air. 3T3-L1 adipocytes were differentiated while referring to the method described previously (Rubin et al., 1977). Briefly, preadipocytes were grown to confluence after which they were cultured for 3 days in DMEM-H, 10% FBS, and antibiotics (culture medium) further supplemented with 10 μg/ml insulin, 0.5 mM isobutylmethylxanthine and 0.25 μM dexametazone.

The cells had accumulated fat droplets after an additional 3 days in the culture medium with 5 μg/ml insulin followed by 3–6 days in culture medium. All stimulations were carried out in DMEM-H without any additions.

2.3. Preparation of RAW264.7-conditioned medium (CM)

RAW264.7 cells were cultured in DMEM-L with 10% FBS in 5% CO_2/95% humidified air at 37 °C. The cells were treated with or without K-111 (30 μM) and/or SP600125 (10 μM) and then were incubated for 18 h, followed by the addition of LPS (1 μg/ml). After 8 h, the medium was changed with or without K-111 (30 μM) and/or SP600125 (10 μM). After 18 or 36 h, the medium was collected and centrifuged at 300 ×*g* for 5 min. The supernatant was concentrated by Centoricon (MILLIPORE Corporation, Billerica, MA, USA), and then was sterilized by filtrating through a 0.22 μm filter, and used as RAW264.7-CM.

2.4. Cytokine production assay using ELISA

RAW264.7 cells suspended in DMEM-L with 10% FBS were placed in multi-well culture plates, and treated with K-111 (30 μM), rosiglitazone (30 μM) or SP600125 (10 μM) followed by the addition of LPS (1 μg/ml). The mixture was incubated at 37 °C for 8 h. The culture medium was then subjected to centrifugation at 300 ×*g* for 5 min, and the concentrations of the cytokines in the supernatant were measured using commercial ELISA kits according to the manufacturer's instructions.

2.5. Measurement of mRNA levels using quantitative real-time reverse transcriptase-polymerase chain reaction

IL-6 and TNFα mRNA expression was determined by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated from RAW264.7 cells or 3T3-L1 adipocytes with ISOGEN (Nippon gene, Tokyo, Japan) and 1 μg RNA was reverse transcribed. The amplification of each target cDNA was performed with TaqMan PCR reagent kits in the ABI PRISM 7700 sequence detection system according to the protocols provided by the manufacturer (PE Applied Biosystems, Foster City, CA). The primer/probe sets of IL-6 and TNFα were purchased from the manufacturer (PE Applied Biosystems, Foster City, CA), and then were used for the amplification step. IL-6 and TNFα mRNA expressions were calculated relative to 18S ribosomal RNA (rRNA).

2.6. Preparation of cytosol extracts

RAW264.7 cells suspended in DMEM-L with 10% FBS were placed in 6-well plates (2 ml/well), and incubated at 37 °C in the absence or presence of K-111 (30 μM). After 30 min, each well