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Activation of a retinoic acid receptor pathway by thiazolidinediones induces production of vascular endothelial growth factor/vascular permeability factor in OP9 adipocytes

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ABSTRACT

Thiazolidinediones, ligands of peroxisome proliferator-activated receptorγ (PPARγ), are used in the management of type 2 diabetes mellitus. However, they can cause edema, which often leads to a discontinuation of treatment. The mechanism by which thiazolidinediones induce edema is poorly understood. We have confirmed that troglitazone (TGZ), a thiazolidinedione, induced the differentiation of a preadipocyte cell line, OP9, into adipocytes. The differentiated OP9 cells produced vascular permeability factors and the activity was completely neutralized by an antibody against vascular endothelial growth factor (VEGF). TGZ induced the expression of VEGF but not interleukin-6 and monocyte chemoattractant protein-1. 2-chloro-5-nitrobenzanilide (GW9662) blocked both the differentiation and the production of VEGF induced by TGZ. 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2, a natural ligand of PPARγ, and another PPARγ agonist, ginkgolic acid, also induced an increase in the expression of VEGE as well as the differentiation of OP9 cells. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) with PPARγ activity, up-regulated VEGF expression, but acetylsalicylic acid, a NSAID without PPARγ activity, did not. Although VEGF expression was enhanced under hypoxic conditions, the expression of hypoxia inducible factor and Ets-1 was down-regulated during the TGZ-induced differentiation. On the other hand, retinoic acid enhanced the expression of VEGF despite inhibiting the TGZ-induced differentiation. Moreover, retinoic acid receptor (RAR) β expression was increased by TGZ and retinoic acid. These findings suggested that the major adipocyte-derived vascular permeability factor produced in response to TGZ was VEGF, and a RAR pathway was involved in the production.

1. Introduction

The thiazolidinediones ameliorate peripheral and hepatic insulin resistance and are effective glucose-lowering treatments for patients with type 2 diabetes mellitus. The American Diabetes Association and the European Association for the Study of Diabetes recommend thiazolidinediones as second- or third-line therapy in combination with other oral agents or insulin to achieve target levels of glycemic control. Thiazolidinediones are ligands of peroxisome proliferator-activated receptorγ (PPARγ), which plays important roles in the differentiation of adipocytes via transcriptional regulation of adipocyte-specific genes (Rosen et al., 1999). PPARγ agonists increased the number of small adipocytes in Obese Zucker rats probably by enhancing the differentiation process (Hallakou et al., 1997).

The risk-benefit ratio for thiazolidinediones use has been the subject of intense discussion following a series of metabolic and cardiovascular outcome studies. Several analyses have highlighted the major adverse effects of thiazolidinediones, including an increased incidence of bone fractures, edema, increased risk of congestive heart failure, increased risk of bladder cancer (Karalliedde and Buckingham, 2007; Cariou et al., 2012) and, more recently, diabetic macular edema (Idris et al., 2012). In particular, fluid retention and edema often lead to a discontinuation of treatment. The meta-analysis provides evidence that thiazolidinediones therapy is associated with at least a two-fold increase in the risk for developing edema (Berlie et al., 2007). The edema is associated with PPARγ-dependent fluid retention through not only non-genomic stimulation of renal salt absorption in proximal tubules (Endo et al., 2011) but also up-regulated expression of the epithelial Na^+ channel in the collecting ducts (Guan et al., 2005).

Another possible mechanism of the edema is the production of vascular permeability factors. Vascular endothelial growth factor (VEGF) is an angiogenic and vascular permeability factor (Leung

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et al., 1989; Keck et al., 1989). VEGF increases vascular permeability a thousand times more potently than histamine (Dvorak et al., 1992; Collins et al., 1993). In addition, VEGF is an important factor for diabetic macular edema (Nguyen et al., 2006).

Previous studies suggested that thiazolidinediones stimulate VEGF production in several types of cells including mature adipocytes in vitro (Yamakawa et al., 2000; Bamba et al., 2000; Emoto et al., 2001). However, there is no binding site for PPARγ in the promoter region of VEGF (Shima et al., 1996). Moreover, it has not been clarified whether VEGF is responsible for the vascular permeability factor produced by adipocytes stimulated by thiazolidinediones. In this study, we examined whether VEGF was the main vascular permeability factor produced in response to thiazolidinediones and how thiazolidinediones induced the production.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs were provided by Japan SLC (Shizuoka, Japan). The guinea pigs were housed with free access to water and food at 22 °C and 55% humidity with lights on from 7:00 to 19:00. All experiments were approved by the local Animal Ethics Committee.

2.2. Cell culture

OP9 mouse stromal cells were obtained from Riken Bio Resource Center (Ibaraki, Japan) and maintained in MEM-α (Invitrogen, Carlsbad, CA) containing 20% (v/v) fetal bovine serum (Invitrogen) and antibiotic–antimycotic (Invitrogen) at 37 °C under 5% CO_2, 95% air. Confluent OP9 cells were treated with troglitazone (TGZ, Sigma-Aldrich, St. Louis, MO), 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2 (15d-PGJ_2, Enzo Life Science, Farmingdale, NY), ginkgolic acid (Kishida Chemical, Osaka, Japan), indomethacin (Wako Pure Chemical, Osaka, Japan), acetylsalicylic acid (Wako Pure Chemical), 9-*cis*-retinoic acid (9CRA, Wako Pure Chemical), or *all-trans*-retinoic acid (ATRA, Wako Pure Chemical) with or without 2-chloro-5-nitrobenzanilide (GW9662, Sigma-Aldrich). In order to induce hypoxia, the cells were incubated in serum-free medium with 250 μM cobalt chloride ($CoCl_2$, Wako Pure Chemical).

2.3. Adipocyte differentiation assay

OP9 cells were incubated in medium containing PPARγ agonists at various concentrations for several days. After the incubation, the cells were washed gently with phosphate-buffered saline (PBS, Wako Pure Chemical), and then lipid droplets were stained and the fluorescence was measured using AdipoRed™ Assay Reagent (Lonza, Basel, Switzerland).

2.4. Real-time reverse transcription-polymerase chain reaction

Total RNA was extracted with ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The total RNA was reverse transcribed using the PrimeScript® RT reagent Kit (Takara Bio, Shiga, Japan). The following primers were used: TEM-1-Forward, 5′-TATCCAGACCTGCCTTTTGG-3′. TEM-1-Reverse, 5′-GGTATCCCCAGGATCAAGGT-3′. Adiponectin-Forward, 5′-AAGGACAAGGCCGTTCTCT-3′. Adiponectin-Reverse, 5′-TATGGGTAGTTGCAGTCAGTTGG-3′. Perilipin-Forward, 5′-AGAGTTCTGCAGCTGCCTGTG-3′. Perilipin-Reverse, 5′-CAGAGGTGCTTGCAATGGGCA-3′. VEGF-Forward, 5′-CTGGCTTTACTGCTGTACCTC-3′. VEGF-Reverse, 5′-CATGGTGATGTTGCTCTCTGAC-3′. MCP-1-Forward, 5′-CCTGTCATGCTTCTGGGCCTGC-3′. MCP-1-Reverse, 5′-GGGGCGTTAACTGCATCTGGCTG-3′. IL-6-Forward, 5′-GAAAAGAGTTGTGCAATGGCAA-3′. IL-6-Reverse, 5′-TCATGTACTCCAGGTAGCTATGG-3′. HIF-2-Forward, 5′-CCTGCAGCCTCAGTGTATCA-3′. HIF-2-Reverse, 5′-GTGTGGCTTGAACAGGGATT-3′. Ets-1-Forward, 5′-GTTTCACAAAAGAACAGCAGCG-3′. Ets-1-Reverse, 5′-TTTCTGTCCACTGCCGGG-3′. RARα-Forward, 5′-ACGAGTCTCCCTGGACATTG-3′. RARα-Reverse, 5′-TTGAGGAGGGTGATCTGGTC-3′. RARβ-Forward, 5′-ATGAATAACCAGGCCTCACG-3′. RARβ-Reverse, 5′-GCAAGGAGAAGCTTCCACAC-3′. RARγ-Forward, 5′-CCACCAAATGCATCATCAAG-3′. RARγ-Reverse, 5′-ATCCGCAGCATTAGGATGTC-3′. GAPDH-Forward, 5′-AGGTCATCCATGACAACTTTGG-3′. GAPDH-Reverse, 5′-CAGTGAGCTTCCCGTTCAG-3′. 18s-Forward, 5′-TTGACGGAAGGGCACCACCAG-3′. 18s-Reverse, 5′-GCACCACCACCCACGGAATCG-3′. The cDNA

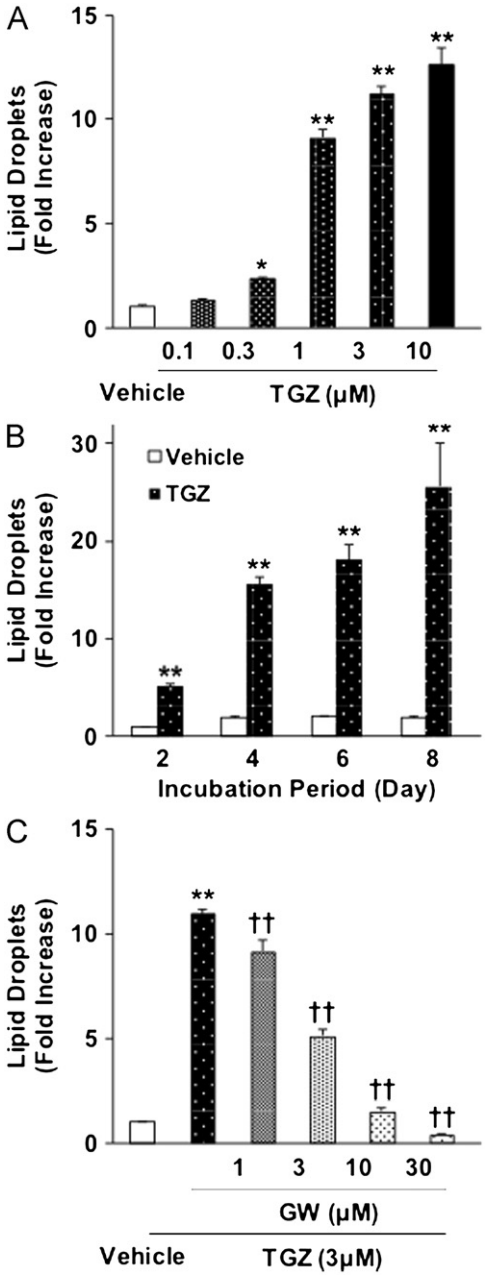

Fig. 1. TGZ induced OP9 cells to differentiate into adipocytes in a PPARγ-dependent manner. OP9 cells were incubated in medium containing the indicated concentrations of TGZ for 6 days (A), TGZ (3 μM) for the periods indicated (B), or TGZ (3 μM) in the presence of GW9662 at the indicated concentrations for 6 days (C). Accumulation of the lipid droplets was examined (n=4–6). The value for the vehicle-treated group (A) and (C) or at 2 days (B) was set to 1. Vertical bars represent the S.E.M. Statistical significance: $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle group, ††$P<0.01$ vs. TGZ alone group. TGZ, troglitazone; GW, GW9662.