ELSEVIER

Cell Biology International 31 (2007) 703–710

Cell Biology International

www.elsevier.com/locate/cellbi

Thiazolidinediones exhibit different effects on preadipocytes isolated from rat mesenteric fat tissue and cell line 3T3-L1 cells derived from mice

Hitoshi Mineo [a,*], Chikako Oda [a], Hideyuki Chiji [b], Teruo Kawada [c], Kyoko Shimizu [a], Toshio Taira [a]

[a] *Primary Cell Co., Ltd., Center for Advanced Science and Technology 306, Hokkaido University, Kita-21 Nishi-11, Sapporo, Hokkaido 001-0021, Japan*

[b] *Faculty of Human Life Science, Fuji Women's University, Hokkaido 061-0067, Japan*

[c] *Division of Food Science and Biochemistry, Graduate School of Agriculture, Kyoto University, Kyoto 105-0001, Japan*

Received 1 November 2006; revised 18 December 2006; accepted 9 January 2007

Abstract

The effects of PPAR-γ agonists, thiazolidinediones (TZDs), on preadipocytes isolated from rat mesenteric adipose tissue and murine cell line 3T3-L1 were compared using an *in vitro* cell culture system. After each cell formed a confluent monolayer under appropriate medial conditions, pioglitazone or troglitazone was applied at 10 μM to each medium for cell maturation. We observed morphological changes in each cell, especially the accumulation of lipid droplets in the cytoplasm, during the culture periods. At the end of culture, DNA content, triglyceride (TG) content and glycerol-3-phosphate dehydrogenase (GPDH) activity were determined. Adiponectin concentrations in each culture medium were also measured during appropriate experimental periods. Application of TZDs increased the DNA content, TG accumulation and GPDH activity in the 3T3-L1 cells but not in the mesenteric adipocytes. Although TG accumulation was unchanged, the number of lipid particles was decreased and the size of lipid particles in the mesenteric adipocytes was increased by TZD application. Although the TZDs increased adiponectin release from the 3T3-L1 cells, adiponectin release from mesenteric adipocytes was suppressed ($P < 0.05$). Thus, the effects of TZDs differed between the primary culture of mesenteric adipose cells and the line cell culture of 3T3-L1 cells. The source of adipocytes is an important factor in determining the action of TZDs *in vitro*, and particular attention should be paid when evaluating the effect of PPAR-γ agonists on adipose tissues.

Keywords: Thiazolidinediones; TZD; PPAR-γ; Cell culture; Adipose tissue; Fat tissue; Rats; Obesity

1. Introduction

The major function of adipose tissue is to store excess energy as neutral fat during periods of nutritional excess (Flier, 1995). These stored fats are used by the organism as metabolic energy during periods of nutritional deficiency, such as starvation. Recently, obesity has become a prevalent health problem in Western countries. Triglyceride (TG) has been shown to be the main fat related to obesity (Ross et al., 1993) and excess accumulation in visceral (omental and mesenteric) adipose tissue was shown to lead to subsequent metabolic syndromes (Matsuzawa et al., 1999; Wajchenberg, 2000). The delivery of free fatty acids to the liver from visceral adipose tissue may contribute to pathological symptoms such as hyperinsulinemia, hypertriglycemia and glucose intolerance (Kissebah, 1991; Bjorntorp, 1997). To reveal the mechanism of differentiation and proliferation of visceral adipocytes is an important problem in the fields of both basic science and applied medicine.

* Corresponding author. Tel./fax: +81 11 706 7325.
E-mail address: mineo@primarycell.com (H. Mineo).

1065-6995/$ - see front matter © 2007 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2007.01.002

Adipocyte differentiation, which consists of various complex processes regulated by a range of factors, has been reviewed in some reports (Gregoire et al., 1998; Rangwala and Lazar, 2000). The transcription process begins with the binding of endogenous or exogenous ligands to encode peroxisome proliferator-activated receptor-γ (PPAR-γ), which is a member of the ligand-activated nuclear receptor family (Kota et al., 2005). A cascade of gene transcription processes then occurs during differentiation, resulting in the expression of adipocyte-specific genes (Tontonoz et al., 1995; IJpenberg et al., 1997). Thus, PPAR-γ is involved in various adipose tissue functions during the adipocyte maturation process.

Thiazolidinediones (TZDs) are representative exogenous PPAR-γ agonists that induce differentiation and proliferation from preadipocytes to mature adipocytes (Furnsinn and Waldhausl, 2002). During the adipocyte maturation process, the uptake of glucose and fatty acids, storage of TG and production of adiponectin were also found to be promoted via TZD-induced activation of PPAR-γ (Spiegelman, 1998; Yki-Jarvinen, 2004). One TZD, pioglitazone, increases the weight of subcutaneous fat tissue through the up-regulation of genes facilitating adipocyte lipid storage *in vivo* (Bogacka et al., 2004). TZDs have been used as anti-type 2 diabetes drugs via their direct effect on fatty acid accumulation in adipocytes, disappearance of glucose and/or their indirect effect on adiponectin release, resulting in lowered insulin sensitivity in outside adipose tissue (Spiegelman, 1998; Yki-Jarvinen, 2004). To clarify the effect of TZDs on adipocytes, it is necessary to evaluate the response to TZDs by various cell types and to recognize differences in cell derivation, such as primary culture cells or cell lines.

The aim of the present study was to compare the effects of TZDs between primary cultured mesenteric adipocytes derived from rat mesentery and line cell 3T3-L1 cells derived from the murine fetus. The cell line 3T3-L1 cells (Green and Meuth, 1974) have been used widely as a representative experimental model for the effects of TZDs on adipose tissues. We observed morphological changes in each cell, particularly the accumulation of lipid droplets in the cytoplasm, during the culture periods and evaluated cell differentiation and proliferation parameters such as DNA content, TG accumulation in the cytoplasm and adiponectin production. We also determined glycerol-3-phosphate dehydrogenase (GPDH) activity, which promotes the conversion of glycerol-3-phosphate to triacylgricerol in the 2 types of cultured cells in the presence of the two TZD drugs.

2. Materials and methods

2.1. Reagents

Two types of TZD, pioglitazone and troglitazone were purchased from Takeda Pharmaceutical Co. Ltd. (Osaka, Japan) and Sankyo Co. Ltd. (Tokyo, Japan), respectively. Hanks' balanced salt solution (HBSS), Dulbecco's modified Eagle/F-12 medium (DMEM/F12), newborn calf serum (NCS) and Trypsin-EDTA solution were purchased from Invitrogen Corporation (USA). Collagenase (Type II), BSA (Fraction V), 3-Isobutyl-1-Methylxanthine (IBMX), Dexamethasone (DEX), and fetal calf serum (FCS) were obtained from Sigma (USA). Other biochemical grade reagents were purchased from Wako Pure Chemical (Osaka, Japan).

2.2. Animals and feeding conditions

The animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals. The cages were placed in a room with controlled temperature (21–23 °C) and lighting (light 0800–2000 h). All animals had free access to tap water and a solid laboratory diet (CE-2, Japan Clea, Tokyo, Japan) during feeding periods before tissue collection. Male Sprague–Dawley rats at 3 to 5 weeks of age were used for the collection of preadipocytes in the mesentery to be used as visceral adipose tissue.

2.3. Cell culture protocol

Fig. 1 shows the experimental protocols for the culture of mesenteric preadipocytes and 3T3-L1 preadipocytes. To induce cell differentiation, the application of promoters (DEX, insulin and/or IBMX) was required for the 3T3-L1 cultures. On the other hand, no specific promotion treatment was needed to induce cell differentiation in the mesenteric adipocyte culture, since insulin was added to the medium during the experimental period. The TZDs were added to the medium immediately after formation of a confluent monolayer in each cell culture system; mesenteric adipocytes at 4 days and 3T3-L1 cells at 14 days. The time course from preadipocyte harvest to mature cell collection at the end of experiment was 13 days for mesenteric adipocytes and 23 days for 3T3-L1 cells.

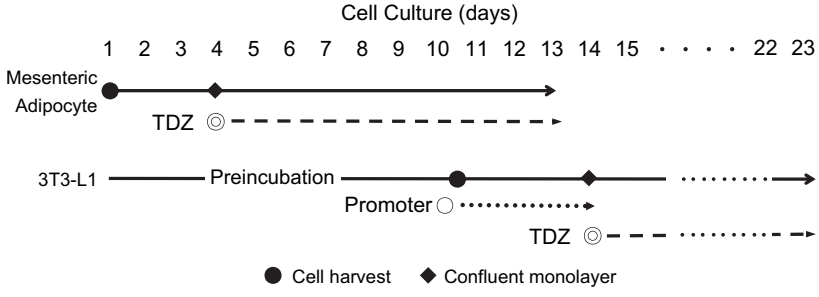

Fig. 1. Experimental protocol for mesenteric adipocyte and 3T3-L1 culture systems *in vitro*. Mesenteric preadipocytes were harvested on incubation plates at Day 1. Specific treatment for the promotion of cell differentiation was not undertaken for the mesenteric adipocyte culture, since insulin (10 μg/ml) was added to the medium throughout the experimental period. Troglitazone or pioglitazone was added at 10 μM from Day 4 to the end of the experiment. Preadipocytes of 3T3-L1 were pre-incubated in a flask from Day 1 to Day 9, and then harvested on incubation plates at Day 10. Differentiation of 3T3-L1 cells was induced by 1 mg/ml IBMX, 1.5 mg/ml DEX and 0.2 μM insulin from Day 10 to Day 12, followed by treatment with 0.2 μM insulin from Day 12 to Day 14. Pioglitazone or troglitazone was added at 10 μM from Day 14 to the end of experiment. The medium was changed daily or every 2 days throughout the experimental periods. The mature mesenteric adipocytes and 3T3-L1 cells were collected at Day 10, Day 13 and Day 23.