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Research Article

EFFECT OF TROGLITAZONE ON GLUT4 GENE EXPRESSION IN 3T3-L1 ADIPOCYTE DIFFERENTIATION

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ABSTRACT

Troglitazone is insulin-sensitizing agents which improve hyperglycemia and hyperinsulinemia by increasing insulin responsiveness and/or sensitivity in obese and type 2 diabetic. To investigate whether this compound could increase the adipogenic gene marker, GLUT4, we add troglitazone on common 3T3-L1 adipogenic induction media. The expression was observed by semi quantitative real time PCR using beta actin as reference gene. Furthermore, the 3T3-L1 differentiated cells was stained by oil red O staining to observe the morphological changing during differentiation. In fully differentiated adipocytes, the GLUT4 over expressed in 3T3-L1 treated by troglitazone. These expression was higher than 3T3-L1 cells treated only adipogenic induction media, and without adipogenic media at all. Moreover, the successfully 3T3-L1 differentiated cells also changed, and seen as rounded adipocyte cells with red droplet lipids around the nuclear.

Keywords: Troglitazone, gene, GLUT4, expression, 3T3-L1, adipocyte.

INTRODUCTION

Adipose tissue is considered a key link between obesity and Type 2 diabetes. It is because insulin resistance in type 2 diabetes either at the adipocyte or skeletal muscle levels might contribute to hyperglycemia. So far, pathways related to insulin resistance be studied in cell lines of adipocytes such as murine 3T3-L1 cells[1]. The 3T3-L1 cell line is derived from disaggregated 17-19 day Murine Swiss mouse embryo. It needs adipogenic agents consisted of insulin, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), and fetal bovine serum (FBS), with around 12-14 days to change the 3T3-L1 fibroblast phenotype cells to adipocyte[2,3].

This adipocyte differentiation process is tightly controlled by molecular and cellular mechanisms, including transcriptional factors and extracellular proteins. Many of the genes associated with the differentiation and maintenance of the adipocyte phenotype could be involved in type-2 diabetes and obesity. Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear receptor superfamily of ligand-inducible transcription factors and is a master regulator of adipocyte differentiation and metabolism, controlling the gene networks involved in lipid metabolism and glucose homeostasis. PPARγ is the ultimate effector of adipogenesis in a transcriptional cascade that also involves members of the C/EBP (CCAAT enhancer binding protein) transcription factor family. Together, these proteins regulate downstream target genes involved in adipogenesis, include Glucose transporter isoform 4 (GLUT4)[4].

GLUT4, the predominant insulin-responsive glucose transporter isoform, plays a key role in the process of transporting extracellular glucose into insulin-sensitive cells in vivo[5]. This is one of 13 sugar transporter proteins encoded in the human genome that catalyzes hexose transport across cell membranes through an ATP-independent. The GLUT4 is highly expressed in adipose tissue and skeletal muscle[6–8]. Studies on over-express GLUT4 in 33T-L1 adipocytes may be employed as tools to evaluate the effects of antidiabetic upon glucose uptake[1,9].

Troglitazone is insulin-sensitizing agents which improve hyperglycemia and hyperinsulinemia by increasing insulin responsiveness and/or sensitivity in obese, type 2 diabetic, and glucose- intolerant patients. These drug increase peripheral glucose uptake, while decreasing the serum insulin level and gluconeogenesis in rodent models of type 2 diabetes [10].

In present study, we investigate the direct effect of troglitazone on GLUT4 expression in 3T3-L1 adipocytes differentiation. We add troglitazone in induction media cocktail which commonly used in 3T3-L1 adipocyte differentiation, and observed this drug effect on expression of GLUT4 by semi quantitative real time PCR using beta actin as reference gene.

MATERIAL AND METHODS

Materials

Mouse 3T3-L1 fibroblast cells were obtained from Korea Research Institute of Bioscience & Biotechnology, South of Korea.

Reagents

Dulbecco's Modified Eagle's Medium 12 (DMEM-F12), IBMX, Amphotericin B, Isopropanol and Chloroform were obtained from SIGMA ALDRICH, USA. Fetal Bovine Serum (FBS) and Trypsin-EDTA (0.5%), no phenol red were purchased from

GIBCOTM, Penicillin-Streptomycin was purchased from THERMO FISHER SCIENTIFIC, Troglitazone and Human insulin were obtained from SANTA CRUSH, and Dexamethasone was obtained from BIOREAGENT. GENEzol™ Reagent was obtained from BIOLINE, SensiFAST CDNA Synthesis Kit and SensiFAST™ SYBR® No-ROX One-Step Kit were purchased from BIOLINE, Primer PPARγ, Beta actin, and GLUT4 were obtained from INTEGRATED DNA TECHNOLOGIES.

Cell culture and differentiation

3T3-L1 pre-adipocytes were grown and passaged in DMEM-F12 containing 10% FBS and 1% Penicillin Streptomycin (DMEM-F12 complete). For adipocyte differentiation, 2 days' post-confluent cells were placed in two different adipogenic differentiation medium with and without Troglitazone. In common adipogenic differentiation medium I, we followed the protocols described by Zebisch et al. 2012 (Zebisch et al. 2012) (DMEM-F12 complete with 0.25 µM Dexamethasone, 0.5 mM IBMX, and 1 µg/ml insulin). For comparison, we add 5 µM Troglitazone on this common medium. Another 3T3-L1 pre-adipocyte cells were also incubated with only DMEM-F12 as negative control group. After 2 days, each media from those three groups were changed by adipogenic induction medium II (DMEM-F12 complete with only 1 µg/ml insulin). The cells back incubated again for two days, and replace with only DMEM F-12 complete per 2 days until day 12.

Oil Red O staining

During differentiation time, morphological changing was observed by Oil red O staining. Sampling has been done three time, first before induction given (day 1), second at day 4 or one day after induction with media contain Troglitazone, and the last was done at the end of differentiation (day 12). The cells washed with phosphate-buffered saline (PBS) and fixed with 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4 for 15 min at room temperature. Then, it washed 3 times with deionized water. A mixture of Oil Red O (0.6% Oil Red O dye in isopropanol) and water at a 6:4 ratios were layered on the cells for 10 min, followed by photographed and read the cell absorbance on 510 nm.

Quantitative Real Time PCR

Total RNA was isolated using GENEzol™ reagent and reverse transcribed with SensiFAST CDNA Synthesis Kit according to manufacturer's instruction. A cDNA then amplified with PPAR-γ and GLUT4 primers, and β-actin primer as reference gene. Quantitative real time PCR was performed on Eco Ilumina 4.1 system using SensiFAST™ SYBR® No-ROX One-Step Kit in a final volume if 20 µl. The condition of real time PCR were as follows: 45˚C for 10 sec, pre-denaturations at 95˚C for 10 sec, denaturation at 95˚C for 5 sec, annealing at 63˚C for 10 sec, and extension 72˚C for 5 sec. These steps replied for 40 cycles. In addition, a melting curve was built in the temperature range of 55-95˚C at the end of amplification. The primer sequences used in this step were presented in Table 1. All primers were designed by SnapGene and synthesized by Integrated DNA Technologies.

Gene expression and statistical analysis

GLUT4 gene expression was measured by comparative CT method (Δ ΔCT) real time PCR using Eco system instrument. Beta actin expression was used as internal control, and before using Δ ΔCT method for quantification, a validation experiment was performed for primer efficiency investigation. In addition, for statistical analysis, all results are presented as mean ± SEM. Differences between the groups were determined by one-way analysis of variance (NOVA), with Post Hoc comparison by Tukey's Multiple Comparison Test. A value of $P \leq 0.05$ was considered to indicate a statistically significant. All analysis was performed using GraphPad Prism 5.0 for Windows Software.

Table 1: Sequence of GLUT4 and beta actin primers

Type of primers	Sequence
Primer Forward GLUT4	5'- GATTCTGCTGCCCTTCTGTC
Primer Reverse GLUT4	5'- ATTGGACGCTCTCTCTCCAA-3
Primer Forward Beta actin	5'-CTCTGGCTCCTAGCACCATGAAGA-3'
Primer Reverse Beta actin	5'-GTAAAACGCAGCTCAGTAACAGTCCG-3'

RESULTS

Observation to the Oil Red O staining showed there were morphological changing in two 3T3-L1 pre-adipose groups which is differentiated to adipose cells. This changing followed by days after adipogenic induction media was given. In contrast, though any differentiated cells found in cells without adipogenic induction media (negative control) group, the lipid droplet was the least. The treatment with adipogenic induction media with and without Troglitazone made these cells changed to mature round fat cells characterized by red droplet lipids around the nuclear (Figure 1). In addition, the differentiated cells stained with Oil Red O indicated that cells treated with Troglitazone had a highest absorbance level and degree of droplet accumulation compared in all groups. Statistical analysis shown there were significantly different found in each groups ($P \leq 0.05$) (Figure 2).

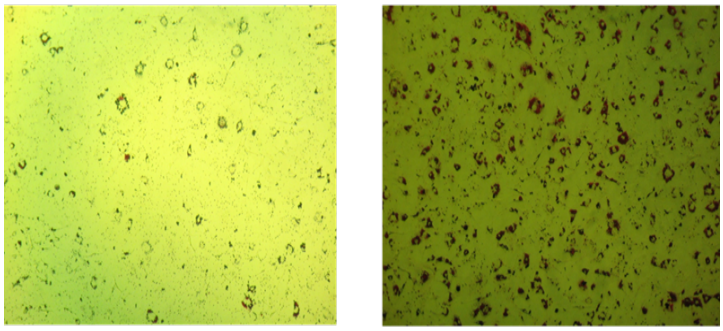

Figure 1: Oro staining result to the cells treated without induction media (left) and induction media plus Troglitazone.

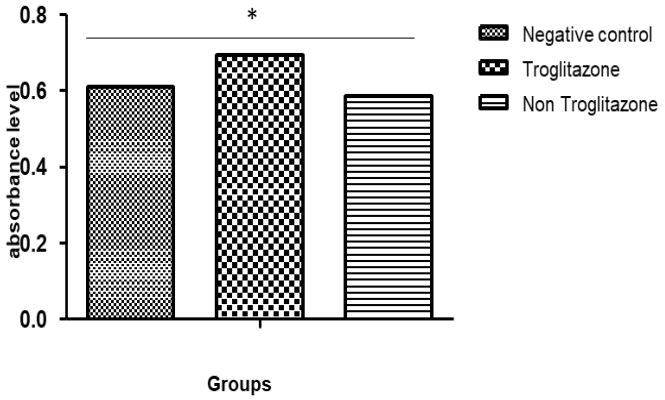

Figure 2: Absorbance level to the 3T3-L1 adipose cells treated with no induction media (negative control), induction media with Troglitazone, and without Troglitazone. *P≤ 0.05 for each groups.

The bar charts showed the comparison of GLUT4 MRNA level on adipocyte cells treated by adipogenic induction media with and without troglitazone, and un-treated with induction media (Figure 3). Generally, troglitazone caused improvement of GLUT4 MRNA level in 3T3-L1 adipocyte cells (fold ct: 1.0). In contrast, no additional of this agent or un-treated at all with induction media didn't cause the escalation of GLUT4 MRNA levels. On the other hand, there were significantly different between troglitazone group and the adipogenic induction media only (p≤ 0.05); and un-treated adipogenic induction media group (p≤ 0.05).

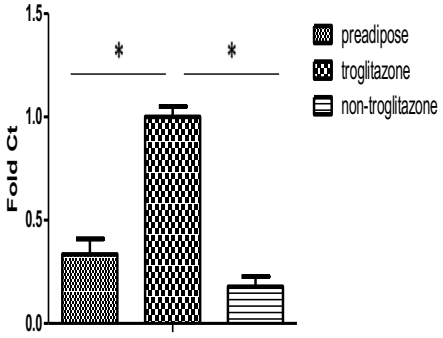

Figure 3: Effect of Troglitazone on GLUT4 MRNA levels in 3T3-L1 adipocytes. Significantly found between troglitazone and non-troglitazone group; and troglitazone and un-treated group *P≤ 0.05.

DISCUSSION

In present study, we observed the effect of Troglitazone on 3T3-L1 pre-adipocytes differentiation and GLUT4 gene expression in 3T3-L1 adipocyte cells. Troglitazone, belong to Thiazolidinediones (TZDs) is antidiabetic which act in improve peripheral insulin sensitivity, leading to reduce blood glucose and insulin levels, and the preservation of pancreatic function [11,12]. Current study found that additional of troglitazone in common adipogenic media was able to stimulate 3T3-L1 pre-adipocyte into adipocyte cells. It's characterized by the size and morphological changes in 3T3-L1 pre-adipocyte. The cells treated by adipogenic induction media with and without Troglitazone were large and had oil droplets were arranged in loose cluster. In contrast, the cells un-treated by adipogenic induction media were small, with compact clusters of oil droplets [13].

Moreover, troglitazone also induce the expression of adipogenesis gene marker, GLUT4 in adipocyte cells. Troglitazone is an agonist ligand to PPAR-γ, a main transcription factors in adipogenesis. Activation of troglitazone to PPAR-γ activate GLUT4 expression which needed to facilitate the uptake of glucose into cells [14,15]. As expected, troglitazone restored the mRNA level of GLUT4 in 3T3-L1 adipocyte cells. This expression was higher in 3T3-L1 troglitazone treated than 3T3-L1 treated only adipogenic induction media, or no adipogenic media. The GLUT4 expression also appear to be dependent during adipocyte differentiation. It's proven by rounded cells with red droplet lipids around the 3T3-L1 adipocyte nuclear cells. Naturally, lipid accumulation (hyperplasia) in adipose tissue is key energy storage organ that regulates whole-body energy homeostasis. Hence, successfully GLUT4 expressed in our study shown that troglitazone potent to regulate glucose homeostasis in adipose tissue. Furthermore, this storing excess energy in the form of triglycerides can be convert into free fatty acids and glycerol to provide energy upon demand in our body [16].

CONCLUSION

In conclusion, we found troglitazone can induce differentiation of 3T3-L1 cells to adipocyte. Its characterized by accumulation of lipid droplets in 3T3-L1 mature adipocyte cells. Moreover, troglitazone also increase GLUT4 expression as adipogenesis gene marker in 3T3-L1 cells.

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REFERENCES

1. Sharma R, Dave V, Sharma S, Jain P, Yadav S. Experimental Models on Diabetes : A Comprehensive Review. *Int J Adv Pharm Sci*. 2013;4:1-8.
2. Zebisch K, Voigt V, Wabitsch M, Brandsch M. Protocol for effective differentiation of 3T3-L1 cells to adipocytes. *Anal Biochem*. 2012;425(1):88-90.
3. Aoyagi R, Funakoshi-Tago M, Fujiwara Y, Tamura H. Coffee Inhibits Adipocyte Differentiation via Inactivation of PPARγ. *Biol Pharm Bull*. 2014;37(11):1820-1825.
4. Chow YL, Iwata Y, Sato F. Dihydrosanguinarine enhances glucose uptake in mouse 3T3-L1 cells. *ACS Omega*. 2017;2(10):6916-6925.
5. Shan W, Chen B, Zhu S, Jiang L, Zhou Y. Effects of GLUT4 expression on insulin resistance in patients with advanced liver cirrhosis. *J Zhejiang Univ Sci B*. 2011;12(8):677-682.
6. Huang S, Czech MP. The GLUT4 Glucose Transporter. *Cell Metab*. 2007;5(4):237-252.
7. Cooke DW, Patel YM. GLUT4 expression in 3T3-L1 adipocytes is repressed by proteasome inhibition, but not by inhibition of calpains. *Mol Cell Endocrinol*. 2005;232(1-2):37-45.
8. G.A. G, G. M. Review on enhancement of glucose uptake and up-regulation of glucose transporters by antidiabetic medicinal plants. *Asian J Pharm Clin Res*. 2016;9(2):34-39.
9. Karthikeyan M, Balasubramanian T, Kumar P. Journal of Developing Drugs In-vivo Animal Models and In-vitro Techniques for Screening Antidiabetic Activity. 2016;5(2).
10. Shintani M, Nishimura H, Yonemitsu S, et al. Troglitazone Not only Increases GLUT4 but Also Induces Its Translocation in Rat Adipocytes. *Diabetes*. 2001;50(10):2296-2300.
11. Camp HS, Li O, Wise SC, et al. Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. *Diabetes*. 2000;49(4):539-547.
12. Alvim RO, Cheuhen MR, Machado SR. General aspects of muscle glucose uptake. 2015;87:351-368.
13. Vishwanath D, Srinivasan H. Novel method to differentiate 3T3 L1 cells in vitro to produce highly sensitive adipocytes for a GLUT4 mediated glucose uptake using fluorescent glucose analog. 2013:129-140.
14. Wong CP, Kaneda T, Morita H. Plant natural products as an anti-lipid droplets accumulation agent. *J Nat Med*. 2014;68(2):253-266.
15. Nugent C, Prins JB, Whitehead JP, et al. Potentiation of glucose uptake in 3T3-L1 adipocytes by PPAR gamma agonists is maintained in cells expressing a PPAR gamma dominant-negative mutant: evidence for selectivity in the downstream responses to PPAR gamma activation. *Mol Endocrinol*. 2001;15(10):1729-1738.
16. Kim JI, Huh JY, Sohn JH, et al. Lipid-Overloaded Enlarged Adipocytes Provoke Insulin Resistance Independent of Inflammation. *Mol Cell Biol*. 2015;35(10):1686-1699.

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