Clinica Chimica Acta 408 (2009) 98–104

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Evidence for an effect of clozapine on the regulation of fat-cell derived factors

Zhi Yang [a,c,1], Ji-Ye Yin [a,1], Zhi-Cheng Gong [b], Qiong Huang [a], Hao Chen [d], Wei Zhang [a], Hong-Hao Zhou [a], Zhao-Qian Liu [a,*]

[a] Institute of Clinical Pharmacology, Hunan Key Laboratory of Pharmacogenetics, Central South University Xiangya School of Medicine, Changsha, Hunan 410078, PR China
[b] Department of Pharmacy, Xiang-Ya Hospital, Central South University, Changsha, Hunan 410008, PR China
[c] Department of Pharmacy, Hunan Children's Hospital, Changsha, Hunan 410007, PR China
[d] Department of Pharmacy, Changsha Central Hospital, Changsha, Hunan 410004, PR China

ARTICLE INFO

Article history:
Received 5 December 2008
Received in revised form 28 July 2009
Accepted 28 July 2009
Available online 11 August 2009

Keywords:
Clozapine
Rosiglitazone
Adipocyte
Fat-cell derived factors

ABSTRACT

Objectives: The aims of this study were to investigate the effects of clozapine on adipocyte differentiation and its regulation for fat-cell derived factors.
Materials and methods: 3T3-L1 preadipocytes were induced into differentiated adipocytes by the addition of 5 μg/ml of insulin, 1 μmol/l dexamethasone, 10 mmol/l IBMX, 1% DMSO, and 10% FBS in DMEM medium. The semi-quantitative RT-PCR was performed to determine the mRNA levels of PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL, and DGAT1. The expression levels of LPL and DGAT1 proteins in the adipocytes treated with clozapine or rosiglitazone were determined by Western blot analysis. The triglyceride concentration was determined by use of GPO-POD assay Kit.
Results: Clozapine enhanced the expression level of ADD1/SREBP$_{1C}$ mRNA and triglyceride concentration in the differentiated adipocytes. Clozapine significantly suppressed the expression levels of LPL mRNA and LPL protein with a dose-dependent and time-dependent manner, respectively.
Conclusion: These data suggest that clozapine might play an important role in inducing adipocyte differentiation and the regulation of fat-cell derived factors.

1. Introduction

Clozapine belongs to one of atypical antipsychotic drugs and it is widely used in the treatment of clinical mental diseases. However, many severe side effects including weight gain and deficiency in granulocyte are observed during the therapeutic proceeding. Among these side effects, weight gain was thought to be a major risk factor of the development of diabetes mellitus, hyperlipidemia, and cardiovascular disease after the patients with psychiatric disease were treated with clozapine for a long time [1]. Up to date, the mechanism of weight gain induced by clozapine is considered to be complicated and remained unknown. Many studies suggest that the possible mechanisms of weight gain are due to poor satiety and increased food intake after the administration of antagonist of 5-HT$_{2C}$ (5-Hydroxytryptamine2C), D2 (Dopamine 2) and H1 (Histamine 1) receptors [2]. Atypical antipsychotic drugs also increased food intake in animal model [3]. At present, most of the studies focused on the contribution of adipocytokines to weight gain. However, the direct pharmacological evidence for weight gain induced by atypical antipsychotic drugs was less understood.

Obesity is a major public health problem in the population. In rodents, obesity is the consequence of an enlargement of adipocyte due to increase of triglyceride accumulation and is characterized by an additional increase of many mature adipocytes [4]. Adipocytes are

Table 1
Primers for the determinations of PPARγ, C/EBPα, SREBP$_{1C}$, LPL, DGAT1, and β-actin mRNA by the use of semi-quantitative reverse transcription polymerase chain reaction (RT-PCR).

Gene	Primers		Length (bp)	T_m (°C)
β-actin	Forward	5′-CACGATGGAGGGGCCGGACTCATC-3′	241	50.5
	Reverse	5′-TAAAGACCTCTATGCCAACACAGT-3′		
PPARγ	Forward	5′-TTATGGGTGAAACTCTGGGA-3′	210	50.5
	Reverse	5′-AATCAACTGTGGTAAAGGGC-3′		
C/EBPα	Forward	5′-CGCAAGAGCCGAGATAAAGC-3′	112	49
	Reverse	5′-CACGGCTCAGCTGTTCCA-3′		
SREBP$_{1C}$	Forward	5′-TAGAGCATATCCCCCAGGTG-3′	245	53
	Reverse	5′-GGTACGGGCCACAAGAAGTA-3′		
LPL	Forward	5′-CTGCTGGCGTAGCAGGAAGT-3′	231	55.7
	Reverse	5′-GCTGGAAAGTGCCTCCATTG-3′		
DGAT1	Forward	5′-TACAGGGAAGAAGGTCAGT-3′	105	46
	Reverse	5′-CACAAAGTAGGAGCAAAGAT-3′		

Abbreviation: PPARγ, peroxisome proliferator-activated receptor-γ; C/EBPα, CCAAT-enhancer binding protein; ADD1/SREBP$_{1C}$, adipocyte determination and differentiation factor 1/sterol regulatory element binding protein; DGAT1, diacylglycerol acyltransferase1; LPL, lipoprotein lipase; PVDF, polyvinylidene difluoride.

* Corresponding author. Tel.: +86 731 4805380; fax: +86 731 2354476.
E-mail address: liuzhaoqian63@126.com (Z.-Q. Liu).
[1] These authors contributed equally.

0009-8981/$ – see front matter.
doi:10.1016/j.cca.2009.07.021

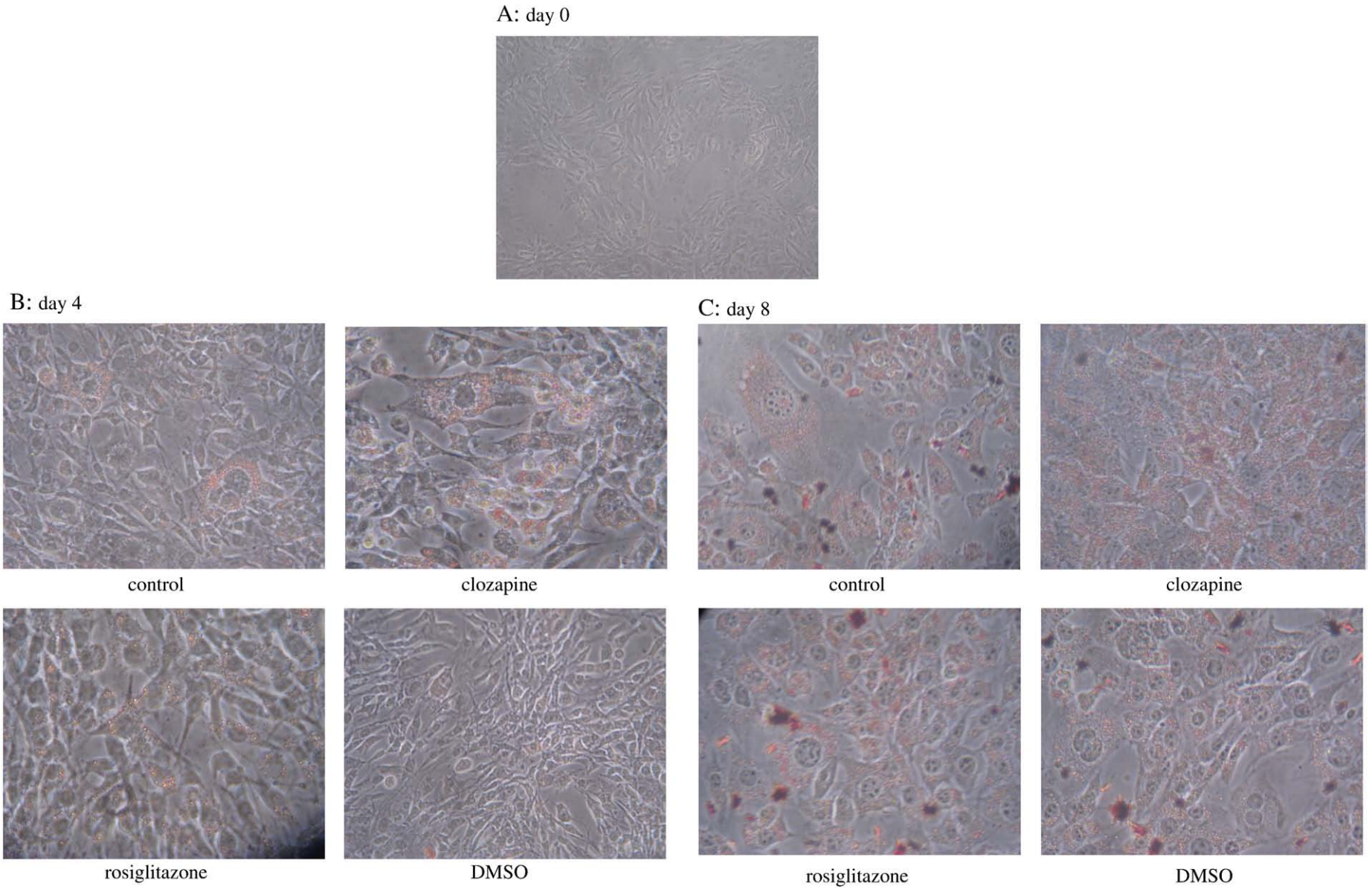


Fig. 1. The photography of the undifferentiated and differentiated 3T3L1 cells stained by red oil. (×100). (A) Cells were stained on day 0; (B) Cells were stained on day 4; (C) Cells were stained on day 8.

Table 2
The percentage of differentiated 3T3L1 cells stained by red oil.

	Control (%)	Clozapine (%)	Rosiglitazone (%)	DMSO (%)
Day 4	24	58	42	20
Day 8	84	100	95	83

derived from mesenchymal precursor cells. Rosen and coauthors found that three key transcriptional factors including peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT-enhancer binding protein α (C/EBPα), and adipocyte determination and differentiation factor 1/sterol regulatory element binding protein (ADD1/SREBP$_{1C}$) play a key role in the adipocyte differentiation [5]. The requirement of PPARγ and C/EBPα in adipose tissue development has been demonstrated by a targeted gene knockout strategy in mice. Homozygous knockout of either gene provokes embryonic lethality and fail to develop normal adipose tissue [6–8]. It was proposed that ADD1/SREBP$_{1C}$ enhances adipose conversion by inducing PPARγ expression [9] and controlling the generation of PPARγ ligands that in turn activate the transcriptional activity of PPARγ [10]. However, the mechanism for ADD1/SREBP$_{1C}$ modulating adipocyte differentiation was not clear.

In the process of adipocyte differentiation and maturation, preadipocyte could be induced into adipocyte after a period of differentiation and some adipocyte-specific proteins related to triglyceride metabolism including diacylglycerol acyltransferase1 (DGAT1) and lipoprotein lipase (LPL) were observed. LPL promotes the formation of triglycerides and is a symbol of adipocyte differentiation [11]. LPL is a key enzyme in decomposition of triglycerides and it plays an important role in the controlling of lipid accumulation [12].

2. Materials and methods

2.1. Cell culture

3T3-L1 cells purchased from ATCC (American Type Culture Collection) (Manassas, VA) were maintained in Dulbecco's modified eagle's medium (DMEM, Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen) before initiating differentiation. Two days after confluence (defined day 0), cells were treated with DMEM containing 10% fetal bovine serum, 1 μg/ml insulin (Sigma, Louis, MO), 0.5 μmol/l dexamethasone (Sigma, Louis, MO, USA), and 1 mmol/l isobutylmethyl xanthine (IBMX, Sigma, Louis, MO) for 48 h. On day 2, cells were fed with DMEM containing 10% fetal bovine serum and 1 μg/ml insulin for 48 h. From day 4, cells were fed with DMEM containing 10% fetal bovine serum alone and then culture medium was changed every 48 h. We added 1, 5, 10, 50, 100 μmol/l of clozapine and 10 μmol/l rosiglitazone to medium to investigate the effect of clozapine and rosiglitazone on the expression of PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL, and DGAT1, respectively. Control cells were treated with DMSO and without any drugs. All cells were collected at days 2, 4, 6, 8.

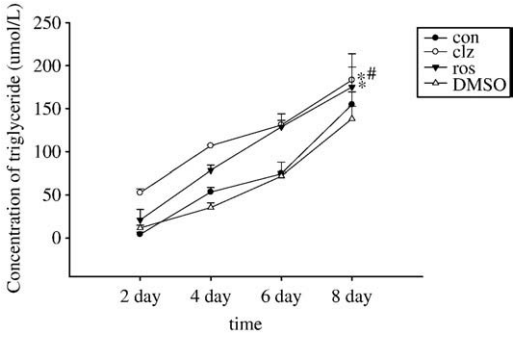


Fig. 2. Effect of clozapine on cellular triglyceride accumulation. *P<0.01 compared with control group and DMSO treatment group, #P<0.01 compared with rosiglitazone treatment group.

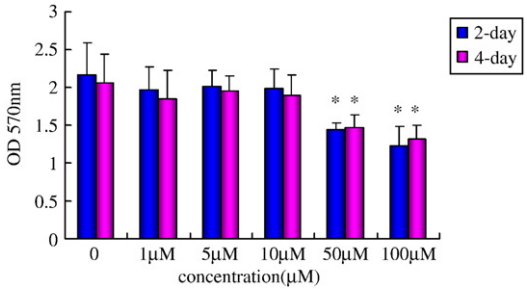


Fig. 3. Effect of clozapine on viability of 2 and 4 days treatment differentiated 3T3-L1 adipocytes by MTT test. *P<0.05 compared with 0, 1, 5 and 10 umol/l, n=3.

2.2. Oil Red O staining

Differentiated 3T3-L1 cells were washed three times with phosphate-buffered saline (PBS) on days 0, 4, 8. Then cells were fixed on dishes with 10% formaldehyde for 1 h. After rinsing with PBS three times, cells were incubated with Oil Red O solution (60% of 0.6% Oil Red O dye in isopropanol and 40% water) for 2 h. Cells were extensively washed with PBS to remove unbound dye. Stained cells were dried on air overnight and then observed under light electron microscope.

2.3. MTT assay

MTT assay is used to test proliferation and viability of culture cells. Clozapine with different concentrations (including 0, 1, 5, 10, 50 and 100 μmol/l) was added to the medium. After 2 and 4 days' treatment, 200 μl MTT solution was added to each well and incubated at 37 °C for 4 h. The incubation was continued for 6 h at 37 °C. The formazan was solubilized by adding dimethyl sulfoxide and the absorbance was measured at 490 nm in Multiskan Ascent 354 microplate reader (Thermo Labsystems).

2.4. Determination of triglyceride in treated cells

Triglyceride concentration was determined by using Triglyceride GPO-POD assay Kit (Applygen, Beijing, China). Briefly, 5–10×10^6 cells/ml PBS was collected and crushed by ultrasonic. 10 μl standard triglyceride or samples and 200 μl working fluid I were mixed together for 5 min at 37 °C, 70 μl working fluid II was added to above mixture solution and then solution was keep at 37 °C for 10 min. Total triglyceride concentration was detected at 490 nm by using multiskan ascend (Thermo, MA).

2.5. RNA isolation and Semi-quantitative RT-PCR

Total RNA was extracted by using trizol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA concentration was determined spectrophotometrically at 260 nm. Genomic DNA from total RNA was removed by using DNaseI (Promega, Madison WI) before reverse transcription. Semi-quantitative RT-PCR was performed as described previously to determine the mRNA levels of β-actin, PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL and DGAT1. The primer pairs of β-actin, PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL and DGAT1 are summarized in Table 1. Polymerase chain reactions (PCR) were carried out using 2 μl cDNA, 2.5 μl 10× standard enzyme buffer (Takara, Dalian, China), 2 μl dNTPs (Takara, Dalian, China), 1 μl of each primer (Biosun, Shanghai, China), and 0.2 units Taq DNA polymerase

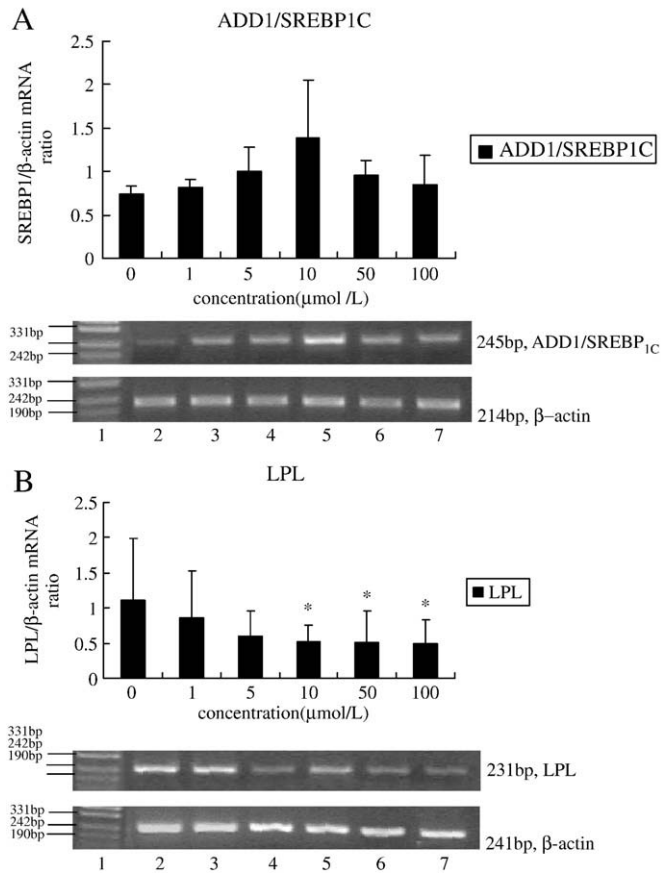


Fig. 4. Effects of clozapine on the expression of ADD1/SREBP$_{1C}$ and LPL mRNA in differentiated 3T3-L1 cells. (A) The dose-dependent effect of clozapine on the expression of ADD1/SREBP$_{1C}$ mRNA. (B) The dose-dependent effect of clozapine on the expression of LPL mRNA. Lane 1 showed DNA marker; Lanes 2 to L7 showed that cells were treated with 0, 1, 5, 10, 50, and 100 μmol/l clozapine, respectively. *P<0.05 compared with the control.

(Takara, Dalian, China). The reaction mixture was made up to 25 μl using water. Samples were heated to 94 °C for 5 min, then amplified through 40 cycles: denaturing at 94 °C for 30 s, annealing at 50.5, 50.5, 49, 53, 55.7, 46 °C for 30 s for β-actin, PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL, and DGAT1, respectively, then extension at 72 °C for 30 s, and a final extension cycle at 72 °C for 5 min.

2.6. Protein preparation and Western blot

Protein samples were prepared as previously described [13]. Briefly, cells were lysed in TNN-SDS buffer (50 mmol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl, 0.5% Nonidet P-40, 50 mmol/l NaF, 1 mmol/l sodium orthovanadate, 1 mmol/l dithiothreitol, 0.1% SDS, and 2 mmol/l phenylmethylsulfonyl fluoride) at 4 °C for 30 min. Lysates were centrifuged at 10,000×g for 5 min at 4 °C and was stored at −20 °C. Protein concentration was determined using Bradford method with Bradford Protein Assay Kit (Beyotime, Shanghai, China) [14].Western blot analyses were performed as described previously with a minor modification [13]. Briefly, cell lysates were separated by 10% SDS-PAGE for 3 h and transferred to a polyvinylidene difluoride (PVDF) membrane followed by a 12 h incubation in 5% non-fat milk in PBST (0.1% Tween20 in TBS) at 4 °C overnight. The blot was then probed with polyclonal antibody sc-32383 for LPL (Santa Cruz Biotech, Santa Cruz CA) and sc-26173 for DGAT1 (Santa Cruz Biotech). The reaction was performed with donkey anti-goat antibody as secondary antibody. The signal was detected by ECM (Boster, Wuhan, China).

2.7. Statistical analysis

All data are expressed as mean ± SD. One-way ANOVA followed by Bonferroni post-tests for multiple comparisons were used to compare means. LSD-t test was used to evaluate differences between groups. Statistical significance was accepted when P<0.05. The SPSS software package (Version 13.0 for Windows; SPSS, Chicago, IL) was used for statistical calculation.

3. Result

3.1. Effect of clozapine on 3T3-L1 cells differentiation

3T3-L1 preadipocytes could be differentiated into mature adipocytes after induction. In this study, we observed the effects of 10 μmol/l clozapine and 10 μmol/l rosiglitazone on 3T3-L1 cell differentiation when preadipocytes were incubated at the different days (Fig. 1, Table 2). No lipid droplets were found on day 0 (Fig. 1A). However, we found that there were a lot of cellular lipid droplets while 3T3-L1 preadipocytes were treated with 10 μmol/l clozapine and 10 μmol/l rosiglitazone at day 4 (Fig. 1B) and day 8 (Fig. 1C). The number of differentiated adipocytes in clozapine treatment group was much more than that in rosiglitazone treatment group and DMSO control.

3.2. Effect of clozapine on cellular triglyceride accumulation

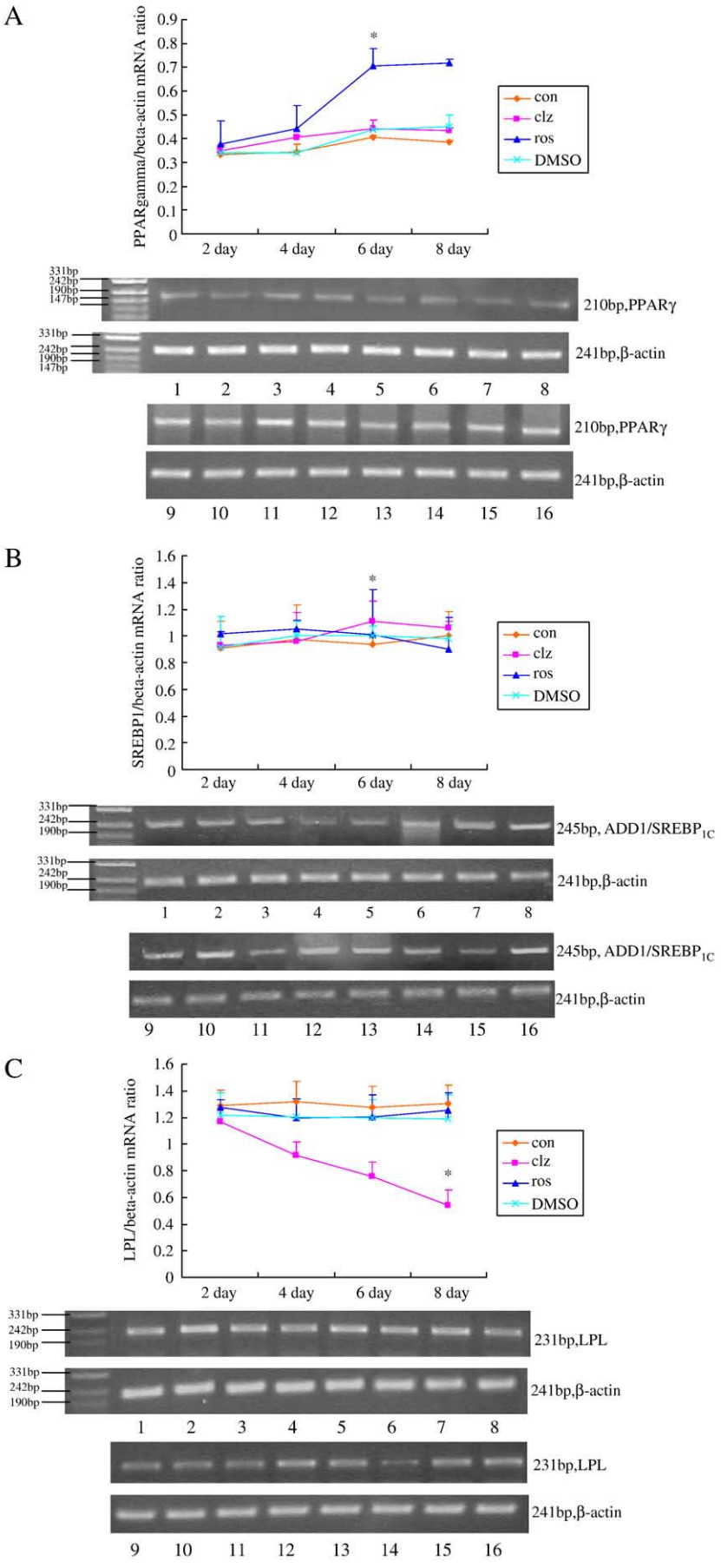
After 3T3-L1 cells were treated with 10 μmol/l clozapine and 10 μmol/l rosiglitazone as well as DMSO for 2, 4, 6, and 8 days, respectively, we found that the triglyceride concentrations in cells are significantly increase while cells were incubated for a longer days. Meanwhile, the concentration of cellular triglyceride in clozapine and rosiglitazone treatment groups were marked higher than that in DMSO treatment group and control group (P<0.001) (Fig. 2), and there are no different between with DMSO treatment group and control group. Finally, we found that triglyceride concentration in clozapine treatment group was higher than that in rosiglitazone treatment groups after cells were treated for 4 days (P<0.01).

3.3. Effect of clozapine on viability of differentiated 3T3-L1 adipocytes

After 2 and 4 days treatment with clozapine at different concentrations, cells were collected to investigate the effect of drug on viability of cells. We found that clozapine does not seem to affect the viability of 3T3-L1 adipocytes *in vitro* after 2 and 4 days treatment at concentrations of 1 to 10 μmol/l, but in 50 and 100 μmol/l, there are statistical significance compare with 10 μmol/l (Fig. 3).

3.4. Effects of clozapine on the mRNA expressions of PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL, and DGAT1

In this study we found that clozapine within the concentration of 1 to 100 μmol/l increased the expression level of ADD1/SREBP$_{1C}$ mRNA with a dose-dependent manner and reached a peak value at 10 μmol/l concentration of clozapine in adipocytes. However, clozapine at the concentrations of 10, 50, and 100 μmol/l significantly decrease LPL mRNA expression in adipocytes (Fig. 4). There were significant differences in the expression level of LPL mRNA between clozapine treatment group and control (10 μmol/l, P = 0.049; 50 μmol/l P = 0.044; 100 μmol/l, P = 0.037, respectively). No significant differences in the effects of clozapine on the mRNA expression levels of of PPARγ, C/EBPα, and DGAT1 were found in this study. Moreover, we found that clozapine at 10 μmol/l slightly increased the mRNA expression levels of PPARγ (Fig. 5A) and ADD1/SREBP$_{1C}$ (Fig. 5B), reached a peak value on ADD1/SREBP$_{1C}$ mRNA for 6 days incubation


A
PPARgamma/beta-actin mRNA ratio
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2 day
4 day
6 day
8 day
*
con
clz
ros
DMSO
331bp
242bp
190bp
147bp
210bp,PPARγ
241bp,β-actin
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
B
SREBP1/beta-actin mRNA ratio
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
245bp, ADD1/SREBP1C
241bp,β-actin
C
LPL/beta-actin mRNA ratio
231bp,LPL

($P<0.05$), and significantly decreased the expression level of LPL mRNA and reached a peak value for 8 days incubation ($P<0.05$) (Fig. 5C). Meanwhile, rosiglitazone at 10 μmol/l significantly increased the expression of PPARγ mRNA (Fig. 5A) reached a peak value for 6 days incubation ($P<0.05$) and decreased the expression of ADD1/SREBP$_{1C}$ (Fig. 5B). Finally, we found that clozapine had no effect on the mRNA expression levels of C/EBPα and DGAT1 in adipocytes. There are no difference between with DMSO treatment group and control group (Fig. 5).

3.5. Effect of clozapine on the protein expression of LPL and DGAT1

As shown in Fig. 6A, we found that clozapine dose-dependently inhibited the expression of LPL protein in adipocytes. There were significant differences in the expression level of LPL protein between clozapine treatment group and control (10 μmol/l, $P=0.001$; 50 μmol/l, $P=0.000$; 100 μmol/l, $P=0.000$, respectively). However, there was no significant difference in DGAT1 protein among different concentrations of clozapine treatment groups.

Clozapine inhibited the expression of LPL protein with a time-dependent manner (Fig. 6B) and the inhibitory magnitude reached a peak value at day 8 ($P<0.01$). However, no significant difference in the expression of LPL protein in adipocytes treated with different concentrations of rosiglitazone was observed in the current study.

4. Discussion

In the previous study, it was shown that several antipsychotic drugs stimulate lipogenesis in cultured cells [15]. In the present study, we observe that clozapine induced the expression of ADD1/SREBP$_{1C}$ mRNA in differentiated adipocytes (Fig. 4A), and also find that clozapine decreased LPL mRNA and protein expression (Figs. 4B and 6A), which suggests that clozapine might induces fat synthesis in adipocytes. These data suggest that clozapine may induce metabolic adverse effects during antipsychotic drug treatment at least in part through direct disturbances of lipid homeostasis in the adipocytes.

Clozapine is an atypical antipsychotic drug exhibiting a low incidence of extrapyramidal side effects and more effective in clinical treatment, so it has been recommended as the first-line drug for the treatment of schizophrenia. Unfortunately, a common side effect of clozapine, namely weight gain, has also been observed. A retrospective analysis showed that the degree of weight gain ranged from 0.04 kg for ziprasidone to 4.45 kg for clozapine was estimated by the random effects regression at 10 weeks [16]. A comprehensive literature analysis revealed that clozapine induced highest weight gain than most other antipsychotics [17].

The mechanism of clozapine-induced weight gain is still unclear. Previously some hypotheses thought that some neurotransmitters such as 5-HT$_{2C}$, D2, and H1 receptors related to feeding behavior and weight regulation were probably involved in the weight gain induced by clozapine. However, whether clozapine can directly promote the differentiation of adipocyte and result in obesity *in vitro*? There were fewer reports about this field and the conclusions are inconsistent. Hemmrich et al. suggested that clozapine could promote preadipocyte differentiation and enhance cellular lipid droplets accumulation [18], but Hauner et al.'s report indicated that there was no evidence for a direct effect of clozapine on fat-cell formation [19].

In our study, we found that clozapine does not seem to affect the viability of 3T3-L1 adipocytes *in vitro* after 2 and 4 days treatment at the concentrations of 1 μmol/l to 10 μmol/l according to the result of MTT. However, there were significant difference between 10 and 50 and 100 μmol/l, this showed that clozapine had toxic effect on adipocyte in 50 and 100 μmol/l.

In this study, we investigated for the first time the regulatory effect of clozapine on the gene expressios of PPARγ, C/EBPα, ADD1/SREBP$_{1C}$, LPL, and DGAT1 in adipocytes. We found that clozapine could increase the expression of ADD1/SREBP$_{1C}$ mRNA with a dosage-dependent manner and a time-dependent manner, but it had no effect on the expressions of C/EBPα and PPARγ mRNA (Figs.4 and 5A). Others have recently demonstrated that many antipsychotic drugs induce transcriptional activation of cholesterol and fatty acid biosynthesis in cultured human cells, including glial cell lines, hepatocytes, and adipocytes [20–22]. Adipose tissue play important roles in energy and lipid metabolism, and we have proposed that antipsychotic-mediated lipogenic effects in peripheral tissues are relevant for the risk of metabolic adverse effects associated with clozapine [20,21]. Our date suggests that the drug-induced ADD1/SREBP$_{1C}$ activation may play an important role in the development of weight gain and metabolic side effects during treatment with clozapine. The drug-induced transcriptional stimulation of cellular lipogenesis is mediated through activation of the ADD1/SREBP$_{1C}$ [20]. In previous some studies, ADD1/SREBP$_{1C}$ was reported to participate in alcohol-induced hepatic steatosis with elevation of cholesterol and triglycerides [23].Changes in ADD1/SREBP$_{1C}$ activity in peripheral tissues can cause pronounced metabolic disturbances, as demonstrated by hepatic steatosis and severe insulin resistance in rodents that over-express ADD1/SREBP$_{1C}$ in liver and in adipose tissue[24–26].ADD1/SREBP$_{1C}$ activation has already been implicated as a possible factor in the development of the metabolic syndrome.

ADD1/SREBP$_{1C}$ is known to interact and cross-talk with other important mediators of metabolic control, such as the transcription regulators PPARs. It has been supposed that ADD1/SREBP$_{1C}$ enhanced triglycerides by inducing PPARγ expression and by controlling the generation of PPARγ ligands [27]. However, our results showed that clozapine had no effect on the expression of PPARγ mRNA, which suggested that the influence of ADD1/SREBP$_{1C}$ on the differentiation of adipocytes was probably not due to the activation of PPARγ or PPARγ ligands. So the effect of clozapine on the expression level of ADD1/SREBP$_{1C}$ protein is necessary in our further study.

The adipocyte plays a crucial role in metabolic regulation, serving as a storage depot for fatty acids and as an endocrine cell to manage energy utilization and feeding behavior [28,29]. The mass of adipose tissue is maintained by a well-controlled balance of cell proliferation (hyperplasia) and increase in fat-cell size (hypertrophy). Increases in adipocyte hypertrophy result from the uptake and assimilation of extracellular fatty acids into cytosolic triacylglycerol-rich lipid droplets. Lipoprotein lipase plays a critical role in the fat metabolism and it is a key enzyme for triglyceride hydrolysis and the transport of free fatty acids [30]. Naturally occurring LPL gene mutations have also been reported in the human population that lead to severe hypertriglyceridemia [31]. In the present study, we found that clozapine could increase the concentration of triglyceride in adipocytes compared with control group (Fig. 2). Meanwhile, we first found that clozapine inhibited the expressions of LPL mRNA and LPL protein with a dosage-dependent manner and time-dependent manner (Figs.4B,5C and 6B). These results suggested that an increase in the accumulation of cellular triglyceride was probably due to the inhibition of expression levels of LPL mRNA and LPL protein.

In conclusion, data from this study suggest that clozapine could induce the expression of ADD1/SREBP$_{1C}$ mRNA and decrease the

Fig. 5. Effects of clozapine on the mRNA expressions of PPARγ, ADD1/SREBP$_{1C}$, and LPL. (A) The time-dependent effect of clozapine on expression of PPARγ mRNA. (B): The time-dependent effect of clozapine on expression of ADD1/SREBP$_{1C}$ mRNA; (C) The time-dependent effect of clozapine on expression of LPL mRNA. *$P<0.05$ compared with two days treatment group. Lanes 1 to L4, L5 to L8, L9 to L12, and L13 to L16 showed that cells were collected on days 2, 4, 6, and 8, respectively. Lanes 1, 5, 9, and 13 represented that cells were treated without any medicines (control); L2, 6, 10, and 14 represented that cells were treated with clozapine; L3, 5, 11, and 15 showed that cells were treated with rosiglitazone; L4, 8, 12, and 16 showed that cells were treated with DMSO.

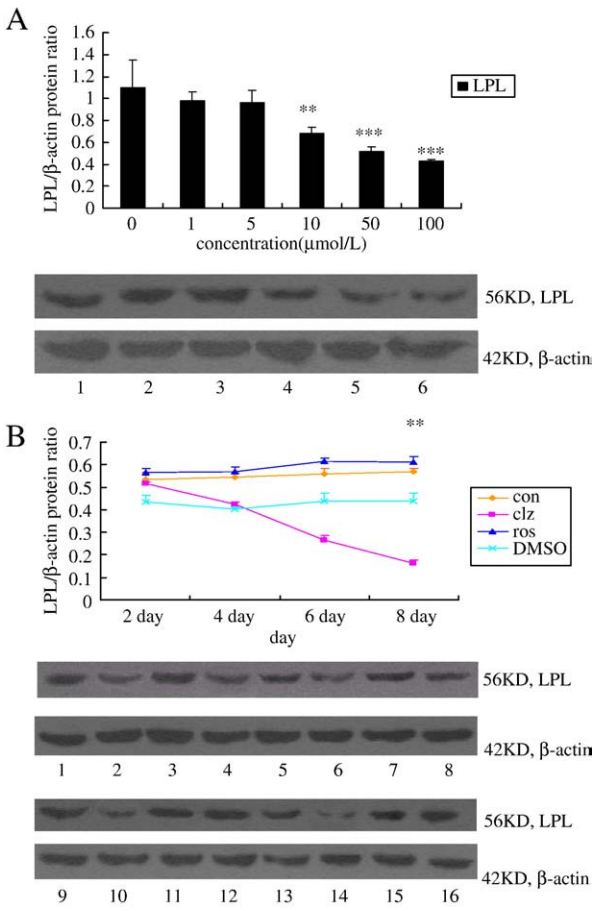


Fig. 6. Effect of clozapine on the expression of LPL protein in differentiated 3T3-L1 cells. (A) The dose-dependent effect of clozapine on expression of LPL protein; (B) the time-dependent effect of clozapine on expression of LPL protein. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ compared with control group.

expression of LPL at the transcriptional and translational levels. The mechanism of weight gain and/or obesity induced by clozapine were possibly due to the suppression of expression levels of LPL mRNA and LPL protein, reduction in triglyceride hydrolysis, and enhancement in lipid droplet aggregation. So we think that clozapine not only influences cell lipogenesis in peripheral but also lipid homeostasis in the whole body and results in weight gain and lipid abnormality.

Acknowledgement

This work was supported by the National Natural Science Foundation of China Grants 30572230, 30873089, by the Hunan Provincial Natural Science Foundation of Grant 08JJ3058, and by the Supported Project for Scientific Technology and Plan of Changsha Government, No. K0802148-31.

References

[1] Nasrallah H. A review of the effect of atypical antipsychotics on weight. Psychoneuroendocrinology 2003;28(Suppl 1):83–96.
[2] Casey DE, Zorn SH. The pharmacology of weight gain with antipsychotics. J Clin Psychiatry 2001;62(Suppl 7):4–10.
[3] Cope MB, Nagy TR, Fernandez JR, Geary N, Casey DE, Allison DB. Antipsychotic drug-induced weight gain: development of an animal model. Int J Obes (Lond) 2005;29:607–14.
[4] Grimaldi PA. The roles of PPARs in adipocyte differentiation. Prog Lipid Res 2001;40:269–81.
[5] Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev 2000;14:1293–307.
[6] Barak Y, Nelson MC, Ong ES, et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol Cell 1999;4:585–95.
[7] Rosen ED, Sarraf P, Troy AE, et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and *in vitro*. Mol Cell 1999;4:611–7.
[8] Wang ND, Finegold MJ, Bradley A, et al. Impaired energy homeostasis in C/EBP alpha knockout mice. Science 1995;269:1108–12.
[9] Fajas L, Schoonjans K, Gelman L, et al. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. Mol Cell Biol 1999;19:5495–503.
[10] Kim JB, Wright HM, Wright M, Spiegelman BM. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proc Natl Acad Sci USA 1998;95:4333–7.
[11] Subauste A, Burant CF. DGAT: novel therapeutic target for obesity and type 2 diabetes mellitus. Curr Drug Targets Immune Endocr Metabol Disord 2003;3:263–70.
[12] Jin W, Marchadier D, Rader DJ. Lipases and HDL metabolism. Trends Endocrinol Metab 2002;13:174–8.
[13] Dong Z, Zhang JT. EIF3 p170, a mediator of mimosine effect on protein synthesis and cell cycle progression. Mol Biol Cell 2003;14:3942–51.
[14] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[15] Vestri HS, Maianu L, Moellering DR, Garvey WT. Atypical antipsychotic drugs directly impair insulin action in adipocytes: effects on glucose transport, lipogenesis, and antilipolysis. Neuropsychopharmacology 2007;32:765–72.
[16] Allison DB, Mentore JL, Heo M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry 1999;156:1686–96.
[17] Muller DJ, Muglia P, Fortune T, Kennedy JL. Pharmacogenetics of antipsychotic-induced weight gain. Pharmacol Res 2004;49:309–29.
[18] Hemmrich K, Gummersbach C, Pallua N, Luckhaus C, Fehsel K. Clozapine enhances differentiation of adipocyte progenitor cells. Mol Psychiatry 2006;11:980–1.
[19] Hauner H, Rohrig K, Hebebrand J, Skurk T. No evidence for a direct effect of clozapine on fat-cell formation and production of leptin and other fat-cell-derived factors. Mol Psychiatry 2003;8:258–9.
[20] Ferno J, Raeder MB, Vik-Mo AO, et al. Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? Pharmacogenomics J 2005;5:298–304.
[21] Raeder MB, Ferno J, Vik-Mo AO, Steen VM. SREBP Activation by antipsychotic- and antidepressant-drugs in cultured human liver cells: relevance for metabolic side-effects? Mol Cell Biochem 2006;289:167–73.
[22] Yang LH, Chen TM, Yu ST. Chen YH Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. Pharmacol Res 2007;56:202–8.
[23] Ji C, Chan C, Kaplowitz N. Predominant role of sterol response element binding proteins (SREBP) lipogenic pathways in hepatic steatosis in the murine intragastric ethanol feeding model. J Hepatol 2006;45:717–24.
[24] Qi NR, Wang J, Zidek V, et al. Kurtz TW A new transgenic rat model of hepatic steatosis and the metabolic syndrome. Hypertension 2005;45:1004–11.
[25] Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS. Goldstein JL Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 1997;99:846–54.
[26] Shimomura I, Hammer RE, Richardson JA, et al. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev 1998;12:3182–94.
[27] Tong Q, Hotamisligil GS. Molecular mechanisms of adipocyte differentiation. Rev Endocr Metab Disord 2001;2:349–55.
[28] Attele AS, Shi ZQ, Yuan CS. Leptin, gut, and food intake. Biochem Pharmacol 2002;63(9):1579–83.
[29] Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord: J Int Assoc Study Obes 1998;22 (12):1145–58.
[30] Buhman KK, Chen HC, Farese RVJ. The enzymes of neutral lipid synthesis. J Biol Chem 2001;276:40369–72.
[31] Ullrich NF, Purnell JQ, Brunzell JD. Adipose tissue fatty acid composition in humans with lipoprotein lipase deficiency. J Investig Med 2001;49(3):273–5.