Acute effects of oral olanzapine treatment on the expression of fatty acid and cholesterol metabolism-related gene in rats

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Abstract
Aims Second-generation antipsychotic drugs (SGAs) have a high risk for serious metabolic side-effects including dyslipidemia. This study aimed to investigate the acute effects of oral olanzapine treatment on the expression of genes for fatty acid and cholesterol biosynthesis in rats. Main methods Female Sprague-Dawley rats were treated orally with olanzapine (1 mg/kg, equivalent to a human clinical dose of 10 mg) via self-administration aimed to measure pharmacokinetics. Based on the pharmacokinetic analysis, the acute effects of olanzapine on sterol regulatory element binding protein (SREBP)-related fatty acid/cholesterol metabolism genes were investigated in the liver and perirenal white adipose tissue (WAT) by Real-time quantitative PCR. Key findings A pharmacokinetic analysis demonstrated that the maximum concentration of olanzapine in plasma (Cmax) occurred at 6 h with a peak concentration of 276.5 ng/ml after a single oral treatment and with a plasma elimination half-life of 3.5 h after peak. The mRNA expression of SREBP-2 and target genes for cholesterol synthesis and transport was increased 1.9-8.8 fold compared with the control at 6 h after olanzapine administration but returned to basal level at 12 h post-treatment, while the increased mRNA expression of SREBP-1c and its targeted fatty acid-related genes appeared at both 6 h and 12 h post-treatment. Significance The present study provided evidence that olanzapine at a clinically-relevant dose caused abnormal expression of genes involved in lipid metabolism in the liver and WAT. These results suggest that olanzapine may cause dyslipidemia side-effects through direct effects on lipid biosynthesis and efflux genes associated with SREBP-stimulated transcriptional changes.

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Title: Acute effects of oral olanzapine treatment on the expression of fatty acid and cholesterol metabolism-related gene in rats

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Highlights

• *In vivo* pharmacokinetics of oral olanzapine treatment in rats

• Olanzapine activates the SREBP-1c-controlling transcription system leading to an increased expression of the key genes involved in fatty acid synthesis

• Olanzapine induces a rapid and transient transcriptional upregulation of genes involved in cholesterol synthesis *via* modulation of the expression of SREBP-2
Abstract

Aims:
Second-generation antipsychotic drugs (SGAs) have a high risk for serious metabolic side-effects including dyslipidemia. This study aimed to investigate the acute effects of oral olanzapine treatment on the expression of genes for fatty acid and cholesterol biosynthesis in rats.

Main methods:
Female Sprague–Dawley rats were treated orally with olanzapine (1 mg/kg, equivalent to human clinical dose of 10 mg) via self-administration aimed to measure pharmacokinetics. Based on the pharmacokinetic analysis, the acute effects of olanzapine on SREBP-related fatty acid/cholesterol metabolism genes were investigated in the liver and perirenal white adipose tissue (WAT) by Real-time quantitative PCR.

Key findings:
A pharmacokinetic analysis demonstrated that the maximum concentration of olanzapine in plasma ($C_{\text{max}}$) occurred at 6 h with peak concentration of 276.5 ng/ml after a single oral treatment, with a plasma elimination half-life of 3.5 h after peak. The mRNA expression of SREBP-2 and target genes for cholesterol synthesis and transport was increased 1.9 8.8 fold compared with the control at 6 h after olanzapine administration but returned to basal level at 12 h post-treatment, while the increased mRNA expression of SREBP-1c and its targeted fatty acid-related genes appeared at both 6 h and 12 h post-treatment.

Significance:
The present study provided evidence that olanzapine at a clinically-relevant dose caused abnormal expression of genes involved in lipid metabolism in the liver and WAT. These results suggest that olanzapine may cause dyslipidemia side-effects through direct effects on lipid biosynthesis and efflux genes associated with SREBPs-stimulated transcriptional
changes.

**Keywords** : Olanzapine; Pharmacokinetics; dyslipidemia; SREBPs
**Abbreviations**

SREBPs: sterol regulatory element-binding proteins

SGAs: second-generation antipsychotic drugs

WAT: white adipose tissue

$C_{\text{max}}$: the maximum concentration of olanzapine in plasma

AUC: the area under the concentration-time curve

$kel$: the elimination rate constant

$t_{1/2}$: the elimination half-life

ALT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: alkaline phosphatase

TG: triglyceride

TC: total cholesterol

HDL-C: high density lipoprotein cholesterol

LDL-C: low density lipoprotein cholesterol

$\text{acc}1$: acetyl-CoA carboxylase

$\text{fasn}$: fatty acid synthetase

$\text{scd}1$: stearoyl-CoA desaturase-1

$\text{ldlr}$: the low density lipoprotein receptor

$\text{abca}1$: ATP-binding cassette transporter 1

$\text{soat}$: sterol o-acyltransferase

$\text{hmgcr}$: 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase

$\text{hmgcs}$: 3-hydroxy-3-methylglutaryl-Coenzyme A synthase
1. Introduction

Second-generation antipsychotic drugs (SGAs), such as olanzapine, have been reported to be better tolerated and have improved efficacy in schizophrenia patients; they are widely used as first line therapy to control schizophrenia symptoms and other psychotic disorders (Meltzer 2013). However, olanzapine have the highest risk for serious metabolic side-effects, such as obesity, dyslipidemia, and even diabetes (Allison et al 2009; Deng 2013). These metabolic side-effects increase risk for cardiovascular disease and premature death (Stahl et al 2009).

While much of the literature has focused on severe weight gain and glucose intolerance, the mechanisms underlying SGA-induced dyslipidemia are far from clear. Accumulated evidence demonstrated that the sterol regulatory element binding proteins (SREBPs) and target genes were thought to be one of possible pathways involved in SGA-induced dyslipidemia (Fernø et al 2011; Raeder et al 2006). SREBPs exist as two homologous proteins: SREBP-1 (with the splice variants SREBP-1a and SREBP-1c) and SREBP-2. SREBP-1 is known as a key regulator of fatty acid synthesis and a molecular link between lipid metabolism, insulin action and obesity at the gene regulatory level (Kotzka & Muller-Wieland 2004), while SREBP-2 is the main regulator of cholesterol metabolism (Shimano 2001). Clozapine and olanzapine induce upregulated expression of SREBPs and target genes in several cell lines, primary rat hepatocytes and in vivo 2-week treatment study (Fernø et al 2005; Skrede et al 2012; Yang et al 2007). However, in contrast to the findings, acute studies in female rats found that a single intraperitoneal (i.p.) injection of clozapine (25 or 50 mg/kg) or olanzapine (5 mg/kg) causes an initial upregulation of lipogenic SREBP target genes followed by a marked and sustained downregulation of these genes in the liver and WAT (Fernø et al 2009; Jassim et al 2012). Although the unexpected biphasic expression pattern of lipogenic SREBP target genes may be partly explained by its parallel with the peak serum concentrations of clozapine and short
half-life of these SGAs (Jassim et al 2012), considering the high dosages of clozapine and olanzapine used in these studies, the biphasic expression pattern is more likely a non-physiological activation followed by a compensatory rebound effect. Therefore, it is necessary to investigate the acute effects of these SGAs at a clinical equivalent dosage on the expression of SREBPs and their target genes in relation to lipogenesis/cholesterogenesis in the liver and WAT.

It should also be noted that olanzapine may have different pharmacokinetics between i.p. injection and oral treatment in rats. Since limited information is available regarding the pharmacokinetics of olanzapine in rats, particularly following oral treatment, the pharmacokinetics of a single oral olanzapine treatment at a clinically relevant dose (1 mg/kg; equivalent to 10 mg in humans at 60 kg body weight) was investigated in this study. Based on the pharmacokinetic analysis, the immediate effects of olanzapine (a single oral dose at 1mg/kg) on SREBP-related fatty acid/cholesterol metabolism genes were investigated in the liver and WAT of female rats.

2. Materials and Methods

2.1. Animals and Housing

Female Sprague-Dawley (SD) rats (200-220 g) were obtained from the Animal Resource Centre (South-West Hospital, Chongqing, China). After one week of environmental familiarization, they were housed in individual cages and allowed ad libitum access to water and a standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate and 16% protein) under environmentally controlled conditions (22°C, with light cycle from 07:00-19:00 and dark cycle from 19:00-07:00) throughout the experimental period. Prior to drug treatment,
rats were trained to self-administer a sweet cookie dough pellet (0.3 g, including 30.9% cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals and 1.6% vitamins) without drugs twice daily (b.i.d.) for one week (Deng et al 2012; Lian et al 2013) and were randomly assigned to one of following experiments (n=6/group). All animal experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), with the approval of the Scientific Investigation Board of Southwest University, Chongqing.

2.2. Pharmacokinetic study in rats treated with a single oral dose of olanzapine

2.2.1. Sample preparation and analysis

Six rats were fasted overnight and treated with a single oral dose of olanzapine (1 mg/kg body weight; Eli Lilly, USA) via a cookie dough pill at ~09:00 am. Immediately following dosing, orbital blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h in heparinized tubes, then centrifuged (1000 g, 10 min, 4 °C) to separate the plasma which was stored at -80°C until assay. A 0.1 ml sample of plasma was extracted using 0.5 ml butyl acetate: dichloromethane (4:1) adding 2 μl 10% (m/v) vitamin C. The sample mixture was mixed on a vortex mixer for 1 min, and centrifuged for 10 min (16000 g). The upper organic layer was separated, dried under N₂ flow, and then dissolved again in a total volume of 50 μl of HPLC grade methanol. The olanzapine assay was developed on an Agilent Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm). Mobile phase A was 0.3% triethylamine in water with the pH adjusted to 3.73 by acetic acid, with methanol as mobile phase B (Morin et al 2005). The flow rate of the mobile phase was maintained at 1ml/min, while elution was conducted in gradient mode as shown in Table S1 (available online). The detection was carried out by UV detector at 254 nm (Cui et al 2011).
Standard stock solutions were prepared by dissolving olanzapine in methanol to yield a nominal concentration of 1 mg/ml. The solutions were subsequently further diluted in methanol to produce working standards. Calibration samples of olanzapine (0.1, 0.2, 0.4, 1, 2, 4, 10 and 20 μg/ml) were prepared by spiking blank plasma with appropriate quantities of working standard solutions. Calibration curves in the 0.1-20 μg/ml range were constructed by plotting the ratios of peak area against concentration. The concentrations of olanzapine in the test samples were calculated using regression parameters obtained from the standard curve.

2.2.2. Pharmacokinetic analysis

All pharmacokinetic parameters of olanzapine were calculated from the plasma concentration versus time using the Data Analysis Pharmacokinetic software DAS 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China). A non-compartmental model was used to determine the pharmacokinetic parameters of olanzapine. Maximum concentration (C\text{max}) of the drug in plasma after a single oral dose (1 mg/kg) was calculated from the recorded data. The area under the concentration-time curve (AUC) was calculated by using the log-linear trapezoidal rule. The elimination rate constant (k\text{el}) was derived from a linear regression of the terminal log-linear disposition phase of the concentration-time curve. The elimination half-life (t\text{1/2}) was calculated as ln2/k\text{el} (Aravagiri et al 1999).

2.3. Acute effects of olanzapine on SREBP-controlled genes in relation to lipogenesis and cholesterogenesis

2.3.1. Animal treatment

Twenty-four rats were fasted overnight, and administered a single oral treatment of olanzapine (1 mg/kg) or vehicle (n=12) at ~09:00 am as described above. Based on the findings in the pharmacokinetic study, treated and control rats were sacrificed by carbon
dioxide asphyxiation at 6 h (n=6/group) or 12 h (n=6/group) after drug treatment. The liver and perirenal WAT were collected and frozen in liquid nitrogen immediately followed by storage in a -80 °C freezer until further analysis. Cardiac blood was collected in procoagulant tubes, left at 37°C for 30 min and centrifuged at 1000 g for 10 min. Serum was transferred to pre-cooled Eppendorf tubes immediately after centrifugation and stored at -20 °C.

2.3.2. Measurements of liver enzymes and serum lipids
Liver enzymes, biochemical parameters (the serum levels of TG, total cholesterol, LDL-C and HDL-C) were measured by colorimetric method using an Olympus AU400 chemistry analyser (Olympus, Tokyo, Japan).

2.3.3. RNA extraction, cDNA synthesis, and gene expression analysis
Total RNA of liver or perirenal WAT was extracted using an RNAsimple Total RNA Kit (DP419) (TianGen, China). To avoid amplification of contaminating genomic DNA, RNA samples were digested with DNase I (Takara, Dalian, China). First strand cDNA was synthesized using the cDNA synthesis kit (DingGuo, China). Cycles were set as follows: 60 min at 42°C, and 5 min at 95 °C, 5 min at 4 °C. Quantitative PCR was performed in a Bio-rad CFX Connect™ Real-Time PCR Detection System using 96-well microliter plates with a final volume of 20 μl mix [composed of 2×10⁻³ nM of specific primers (Invitrogen, China), 8 μl of water, 10 μl of the SYBR® GreenER™ qPCR SuperMix from Invitrogen™, and 2 μl of diluted cDNA]. Thermal cycling parameters were 2 min at 95 °C, followed by 40 cycles each containing 30 s at 94 °C, 30 s at 60 °C , and 30 s at 72 °C. Based on the previous report (Skrede et al 2012), primers (see Table S2) (available online) were designed from sequences in the Genbank databases using Primer 5 software. Each primer pair was then validated by an efficacy ≥95%. All samples were analysed in duplicate. In a pre-experiment, all genes
expression levels were normalized relative to two endogenous controls, β-actin (Actb) and glyceraldehyde-3-phosphate dehydrogenase (Gapdh), which showed stable and similar results with both housekeeping genes. Since Gapdh was more strongly expressed and showed the higher level of accuracy in Ct value than Actb in the pre-experiment, Gapdh was chosen as the endogenous control gene in this study. Results were expressed in relative expression using the comparative 2^{-ΔΔCt} method. The mean value of the control group was set at 1 and all data were normalized versus control group.

2.4. Statistical analysis

Data were expressed as mean ± SEM, and all data were analysed using the SPSS program (IBM version 19.0, SPSS Inc., USA). Due to the small sample size (6 rats/group), data were analysed using the Kruskal–Wallis H test, followed by a post-hoc Mann-Whitney U test at each time point. Statistical significance was accepted when p<0.05.

3. Results

3.1. Pharmacokinetic parameters of a single oral dose olanzapine

The calibration curve was linear within the concentration range assayed. The mean regression value of the calibration curve was y = 27.06x -1.2095 (r^2 = 0.9973) for olanzapine. After olanzapine was orally self-administered via cookie dough to the rats, the plasma concentration versus time profile was shown in Fig.1. The C\text{max} occurred at 6 h after oral administration and the elimination half-life (t\text{1/2}) was 3.5 h after the peak. The major pharmacokinetic parameters of olanzapine are listed in Table 1.

3.2. Serum biochemical parameters
Table 2 presents the levels of TG, total cholesterol, HDL-C, LDL-C, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase at 6 h or 12 h after a single oral dose of olanzapine. Compared with the control group, there was only a tendency for increase in liver aspartate aminotransferase levels (115±16%, \(p=0.091\)), while in the plasma lipid there were no significant changes at any time point after a single oral olanzapine treatment.

### 3.3 mRNA expression levels of fatty acid synthesis-related genes in the liver and WAT

As shown in Fig. 2A, there was upregulation of SREBP-1c mRNA expression in the liver at both examined time points compared to controls (2.1-fold at 6 h, and 3.5-fold at 12 h, \(p<0.05\)). Consistent with the alteration of \(srebp-1c\), fatty acid synthetase (\(fasn\)) mRNA expression was significantly higher at the time points in the treatment group after the single oral olanzapine dose (4.7-fold at 6 h and 3.4-fold at 12 h respectively, all \(p<0.05\)). However, there was no significant difference observed between the olanzapine and control groups in the acetyl-CoA carboxylase (\(acc1\)) expression level after drug treatment.

Similar to the liver, \(srebp-1c\), \(acc1\) and \(fasn\) involved in fatty acid biosynthesis were over-expressed in viceral WAT after one oral dose treatment of olanzapine (Figs. 2A and 2B). However, unlike the liver, there was a stronger upregulation of the mRNA expression for these genes at 12 h than at 6 h post-treatment. In particular, the \(srebp-1c\) expression level was significantly increased (up to 9.7-fold) at 12 h \((p<0.01)\). The maximal upregulated response was recorded for the expression of \(fasn\) gene at 12 h post-treatment (11.13-fold, \(p<0.01\); Fig. 2B).

### 3.4 mRNA expression levels of cholesterol biosynthesis and transport-related genes in the liver and WAT
As presented in Fig. 3A, olanzapine affected mRNA expression of SREBP-2 and its target genes, with a significant difference between expression at 6 h and 12 h after oral treatment. The mRNA levels of these genes were significantly increased at 6 h, followed by a sharp decrease, and a return to baseline (the control level) at 12 h. In the observed period, \( srebp-2 \) (3.57-fold increase, \( p<0.05 \)) and its target genes \( hmgcs \) (4.64-fold increase, \( p<0.01 \)) and \( hmgcr \) (8.82-fold increase, \( p<0.05 \)) were significantly stimulated only at 6 h after drug administration when the plasma concentration of olanzapine peaked at 276.5 ng/ml. For ATP-binding cassette transporter 1 (ABCA1), a cholesterol efflux pump in the cellular lipid removal pathway (Schmitz & Langmann 2001), its mRNA was found to be overexpressed in the liver, leading to a 3.48-fold increase (\( p<0.05 \)). For sterol o-acyltransferase (SOAT), a key enzyme in controlling cholesterol absorption, the up-regulated expression of mRNA appeared only at 6 h after drug administration (\( p<0.05 \); Fig.3A).

In visceral WAT from olanzapine-treated rats, similar to the pattern in the liver, a drug-induced increase of cholesterol biosynthesis-related genes was observed only at 6 h post-treatment (Figs. 3A and 3B). The maximal upregulated response was recorded for the expression of \( srebp-2 \) at 6 h post-treatment (10.42-fold; \( p<0.01 \)). However, 12 h after oral treatment of olanzapine, all genes examined returned to the baseline.

**4. Discussion**

In this study, we examined the direct effect of olanzapine (1mg/kg) in inducing transcriptional activation of SREBPs and SREBP-controlled lipid-related genes in female rats after a single oral treatment. Pharmacokinetic analysis showed that the \( C_{\text{max}} \) and elimination half-life of olanzapine were 276.5 ng/ml and 3.5 h, respectively. Further analysis of the gene expression showed that olanzapine activated the SREBP-1c-controlling transcription system,
and then led to a continuous stimulation of the key genes involved in fatty acid synthesis until at least 12 h after treatment, while, a rapid and transient transcriptional upregulation of the genes involved in cholesterol metabolism was observed at 6 h post-treatment and returned to normal via modulation of the expression of SREBP-2 after 12 hours of treatment. Our data provide further evidence that transcriptional deregulation via upregulation of mRNA of SREBPs transcription factors may contribute to olanzapine-induced disturbance in lipid metabolisms.

Although olanzapine oral administration is frequently used in clinics, a majority of previous studies in animals were through intraperitoneal (i.p.) injection (Fell et al 2008; Fernø et al 2009; Jassim et al 2012) or continuous delivery via a minipump (Mann et al 2013; van der Zwaal et al 2008). Neither i.p. injection nor minipump delivery closely mimic clinical oral treatment conditions, because they may have different pharmacokinetics from oral administration in rats. The oral dose of 1 mg/kg olanzapine is equal to about 10 mg in human (60 kg body weight) according to dosage translation between species based on body surface area following the FDA guideline (FDA 2005; Reagan-Shaw et al 2008), and this is among the recommended clinical dosages for treating schizophrenia patients (Kantrowitz & Citrome 2008). Although our previous studies have successfully established a female rat model for olanzapine-induced metabolic side-effects using oral treatment of 1mg/kg olanzapine (Lian et al 2014; Weston-Green et al 2011; Weston-Green et al 2012), to better understand the mechanism of dyslipidemia, it is important in further studies to employ multiple doses for investigating the dose-response effects on gene transcription.

In this study, the maximum plasma concentration was 276.5 ng/ml at 6 hours after one oral administration of 1mg/kg olanzapine in cookie dough. The observation was not consistent
with previous findings in the peak time. In an earlier study, a rapid absorption was observed and the peak appeared within 45 min after an oral gavage of 6 mg/kg olanzapine (Aravagiri et al 1999). In another study, after a single intraperitoneal dose of 5 mg/kg, the serum level of olanzapine peaked at 1 h after injection (Jassim et al 2012). Since olanzapine was prepared with the cookie dough in this study, a most likely explanation for the delay of drug absorption was due to a prolonged release of olanzapine from the cookie mix and/or delayed gastrointestinal absorption (Mauri et al., 2007). Another explanation is that olanzapine could be absorbed faster at the higher dose (5-6 mg/kg) used in previous reports (Aravagiri et al 1999; Jassim et al 2012). In the current study, the volume of distribution and clearance characteristics of olanzapine resulted in an elimination half-life of 3.5 h, which was slightly longer than the 2.5 h reported in male rats by Aravagiri (Aravagiri et al 1999). This is probably because of the differences in metabolic capacity of the cytochrome P450 (CYP450) isoenzyme system between male and female rats. It is well recognized that CYP450 mediated oxidation is the primary metabolic pathway for olanzapine (Azzaro et al 2007; Sheehan et al 2010). Interestingly, clearance of olanzapine has been reported to be approximately 30% higher in men than women, due to the differences in metabolic capacity of CYP450 between males and females (Gervasini et al 2013; Kelly et al 1999). Therefore, elimination half-life of olanzapine in females might be slightly longer than those in males. Finally, based on a 3.5 h elimination half-life of olanzapine, 6-h and 12-h time points after a single oral-administration were chosen to detect the effects of olanzapine on mRNA expression of hepatic and WAT genes.

Olanzapine was observed to induce a direct transcriptional upregulation of SREBPs and its target genes in the liver in the present study. At 6 h post-treatment, olanzapine caused a significant increase in the expression of both srebp-1c and its downstream target genes (e.g.
1 and fasn), as well as srebp-2 and its downstream cholesterogenic genes (e.g. hmgcs,
hmgcr and ldlr). Our current data confirmed the previous in vitro findings of a direct
antipsychotic drug effect on expression of genes including de novo lipogenesis and
cholesterogenesis. Studies in rat primary hepatocytes (Laressergues et al 2010; Oh et al
2011), or liver cell lines of THLE-3 and HepG2 (Raeder et al 2006; Yang et al 2007) have
shown upregulation of the transcription factors srebp-1 and/or srebp-2 after clozapine or
olanzapine treatment. Since olanzapine did not promote significant changes in blood TC, TG,
HDL-C, LDL-C after 6 or 12 hours of a single treatment in this study, these mRNA changes
observed should not be a feedback effect of dyslipidemia. Therefore, these data suggest that
olanzapine-mediated transcriptional upregulation of SREBPs and their target genes might be
a direct treatment effect. Once activated by olanzapine, SREBPs may start to induce de novo
lipogenesis and cholesterogenesis. This could lead to a sequence of steps stimulating an
abnormal production of fatty acid and cholesterol under chronic treatment of olanzapine.

In the present study, responding to olanzapine treatment, transcriptional activation patterns
were different between lipogenic genes and cholesterogenic genes. As shown in Figs. 2 and
3, there was a clear trend in the transient changes in the expression of SREBP-2-controlled
cholesterol metabolism genes following olanzapine treatment: the initial upregulation was
followed by a marked return to the basal level at 12 h after olanzapine treatment,
corresponding with a peak serum concentration of olanzapine at 6 h followed by a rapidly
declining serum drug concentration. In contrast, srebpl and target lipogenic genes such as
fasn and acc1 showed a different expression pattern: an early upregulation was followed by a
sustained activation of transcription at 12 h after olanzapine treatment in both the liver and
visceral WAT (Figs. 2A and 2B), which suggested that the expression of srebp-1 and its
target lipogenic genes was not affected by the decline in serum olanzapine concentration at
12 h post-treatment. However, it is very interesting that a different (biphasic) expression pattern of lipogenic genes and cholesterol metabolism genes was reported previously in female rats: an i.p. injection of olanzapine (5mg/kg) or clozapine (25mg/kg or 50mg/kg) induced an initial upregulation of SREBP-controlled gene expression followed (at around 1 h post-treatment) by a marked downregulation of SREBPs target genes in rats (Fernø et al 2009; Jassim et al 2012). Although the initial upregulated expression of SREBPs target genes may be explained by the parallel peak concentration of serum drug concentration observed in these studies, the following downregulation could not be well explained by the serum drug concentration. Given the high dosages of clozapine and olanzapine used in these studies, the biphasic expression pattern is more likely a non-physiological activation followed by a compensatory rebound effect (Fernø et al 2009; Jassim et al 2012). Therefore, the gene expression patterns observed in the current study are most likely physiological responses to olanzapine treatment at a clinical equivalent dosage. Since srebp-1c and its downstream target fatty acids biosynthesis genes (e.g. acc1 and fasn) showed a consistent expression pattern compared to srebp-2 and its downstream cholesterogenic genes (e.g. hmgcs, hmgcr and ldlr), our findings should be one possible explanation for the clinical feature that dyslipidemia in the form of hypertriglyceridemia occurs more frequently than hypercholesterolemia in patients treated with SGAs (Birkenaes et al 2008; Sadibasic et al 2014).

Our study revealed that the expression of srebp-1, srebp-2 and their target genes in WAT displayed a similar pattern as in the liver after one single oral treatment of olanzapine. Consistently with this, a previous study reported an upregulation of srebp-1 and SREBP-1-related adipogenesis in the 3T3-L1 adipocyte cell line (Yang et al 2007). However, an in vivo study has reported that, following one i.p. injection of olanzapine (5mg/kg), a non-significant
initial upregulation of lipid-related genes was observed in mesenteric WAT, although a significant upregulation was caused in the liver with the same treatment (Jassim et al 2012). Of note, in this study, a greater expression of srebp-1c and fatty acid biosynthesis-related genes (acc1 and fasn) was observed in WAT than in the liver, particularly at 12 h (Fig. 2). It was not clear what factor(s) caused the greater effect on gene expression in WAT. However, several lines of evidence have proved that the WAT played a more important role in de novo lipogenesis than the liver (Pearce 1983; Swierczynski et al 2000). Parasympathetic inputs to adipose tissue modulates glucose uptake and free fatty acid metabolism (Kreier et al 2002) and has selective effects on local hormone synthesis controlling the specific activities of lipogenic enzymes (Fliers et al 2003). Cholinergic muscarinic receptors are present in WAT (Yang et al 2009). Therefore, it is possible that the greater expression of fatty acid-related genes in the WAT was induced by olanzapine through activation of parasympathetic nerves. Although our present knowledge on the regulation of srebp-1 expression in the WTA is far from complete, it has been shown that tissue-specific expression of SREBP-1 is responsible for the differential expressions of lipogenic genes between the liver and adipose tissue (Foretz et al 1999). In addition, the SREBP-1 may play additional roles in adipose tissue. For example, overexpression of SREBP-1 in cultured 3T3-L1 preadipocytes was shown to promote adipocyte differentiation induced by activation of peroxisome proliferator-activated receptor-gamma (PPARγ), a known activator of adipocyte differentiation (Kim & Spiegelman 1996; Sertie et al 2011). Indeed, antipsychotic treatments alter the gene expression patterns in adipocytes in coordinated fashion (Sarvari et al 2014). Hence, it is important to further investigate the roles of SREBP in adipogenesis.

One limitation of this study was that only olanzapine was examined in this study. Interestingly, it was recently demonstrated that clozapine and risperidone elicited significant
inductions of SREBP-1 maturation and FASN mRNA expression in primary cultures of rat hepatocytes and 3T3-L1 cells associated with an increase of triacylglycerol (Hu et al 2010; Lauressergues et al 2011). Therefore, it is also important in further study to compare the effects of these antipsychotics on inducing SREBP-controlled transcriptional activation of lipogenesis in the liver and WAT.

In summary, olanzapine can directly affect the expression of lipid metabolism genes through regulation of SREBP in the liver and WAT. In addition, SGAs have high binding affinities with multiple neurotransmitter receptors, such as the 5-HT_{2C} (5-HT_{2C}R), histamine H_{1} (H_{1}R) and muscarinic M_{3} receptor (M_{3}R) (Correll 2010), which are associated with SGA-induced metabolic side-effects such as weight gain and diabetes (Correl et al 2012; Deng 2013; Nasrallah 2008). Further research is important to identify the role of these peripheric neurotransmitter receptors in SGA-induced dyslipidemia. If these relationships can be fine-tuned, the genes involved in lipid metabolism might be promising targets for pharmacological intervention to control the metabolic-side effects caused by SGAs.

**Supplementary Material**

Supplementary Material includes two tables and can be found with this article at Web version on PubMed Central.

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**Conflict of Interest**

The authors have no conflicts of interest to disclose.
References


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Figure 1

Plasma concentration-time profile (n=6) of olanzapine (mean ± SEM) after administering an oral dose of 1 mg/kg to female SD rats weighing 200-220 g. The parent drug was isolated from plasma by liquid-liquid extraction with butyl acetate: dichloromethane (4:1) and analyzed by HPLC.
Figure 2

The effects of olanzapine on gene expression involved in fatty acid biosynthesis. Relative expression levels of (A) fatty acid biosynthesis genes in the liver, (B) fatty acid biosynthesis genes in the perirenal white adipose tissue (WAT). Total RNA was extracted and gene expressions were quantified using Q-PCR at 6 h and 12 h after a single oral treatment with 1 mg/kg olanzapine. mRNA expression levels were normalized to the expression of housekeeping gene gapdh. Each column is the mean ± SEM (n=6). *, p<0.05; **, p<0.01.
Figure 3

The effects of olanzapine on gene expression involved in cholesterol biosynthesis and transport. Relative expression levels of (A) cholesterol biosynthesis and transport genes in the liver, (B) cholesterol biosynthesis and transport genes in WAT. Total RNA was extracted and gene expressions were quantified using Q-PCR at 6 h and 12 h after a single oral treatment with 1 mg/kg olanzapine. mRNA expression levels were normalized to the expression of housekeeping gene gapdh. Each column is the mean ± SEM (n=6). *, p<0.05; **, p<0.01
Table 1  Pharmacokinetic parameters of olanzapine after a single oral dose treatment in female SD rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Olanzapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/ml)</td>
<td>276.5</td>
</tr>
<tr>
<td>%CV</td>
<td>29.75</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>6.0</td>
</tr>
<tr>
<td>AUC_{0→24} (ng* h/ml)</td>
<td>38578</td>
</tr>
<tr>
<td>AUC_{0→∞} (ng* h/ml)</td>
<td>78636</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>3.5</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>10.213</td>
</tr>
<tr>
<td>Cl/F (ml/h/kg)</td>
<td>206.78</td>
</tr>
<tr>
<td>V_d/F (ml/kg)</td>
<td>2860.13</td>
</tr>
</tbody>
</table>

C\_{max}, peak plasma concentration; t_{max}, time to reach peak plasma concentration; AUC_{0→∞}, area under the concentration–time curve from zero to infinity; AUC_{0→24}, area under the concentration–time curve from zero to the last measurable plasma concentration; t_{1/2}, elimination half-life; MRT, mean residence time; Cl/F, clearance; V_d/F, volume of distribution.
Table 2  Serum lipid levels and other biochemical parameters following olanzapine oral administration to rats

<table>
<thead>
<tr>
<th>Time point</th>
<th>group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>AST/ALT</th>
<th>ALP (U/L)</th>
<th>TG (mmol)</th>
<th>TC (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>Control 1</td>
<td>24.40±1.96</td>
<td>65.40±6.73</td>
<td>2.68±0.15</td>
<td>110.40±3.03</td>
<td>0.50±0.07</td>
<td>1.35±0.06</td>
<td>1.14±0.05</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td></td>
<td>Treatment 1</td>
<td>25.50±1.67</td>
<td>76.50±2.95</td>
<td>3.04±0.15</td>
<td>120.67±11.74</td>
<td>0.51±0.06</td>
<td>1.32±0.13</td>
<td>1.13±0.11</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>12 h</td>
<td>Control 2</td>
<td>29.20±1.91</td>
<td>76.60±4.62</td>
<td>2.64±0.14</td>
<td>99.00±16.47</td>
<td>0.68±0.07</td>
<td>1.51±0.07</td>
<td>1.32±0.06</td>
<td>0.48±0.02</td>
</tr>
<tr>
<td></td>
<td>Treatment 2</td>
<td>31.67±2.81</td>
<td>88.17±12.39</td>
<td>2.75±0.17</td>
<td>117.50±12.06</td>
<td>0.67±0.08</td>
<td>1.40±0.16</td>
<td>1.24±0.14</td>
<td>0.44±0.06</td>
</tr>
</tbody>
</table>

The levels (mean ± SEM) of serum ALT, AST, ALP, TG, TC, HDL-C, and LDL-C were measured in rats exposed to a single oral dose of olanzapine (1 mg/kg). The changes in the amount of lipids were measured at 6 h and 12 h after olanzapine administration (n=6/group). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.