Increased expression of lipid biosynthesis genes in peripheral blood cells of olanzapine-treated patients

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Abstract
Recent in-vitro studies show that antipsychotic drugs increase lipid biosynthesis through changes in gene expression. Based on these findings we compared the expression of two central lipid biosynthesis genes, fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD), in whole blood of olanzapine-treated and unmedicated patients. Patients with psychotic disorders were consecutively selected from an ongoing, naturalistic study, and divided into two groups according to the following criteria: (1) strict monotherapy with olanzapine (n = 19) or (2) no current medication (n = 19). The groups were matched on gender, race and body mass index. Blood lipid levels were examined, and gene expression in whole blood was assessed with quantitative real-time PCR. Expression of FASN (p = 0.003) and SCD (p = 0.002) was significantly up-regulated in olanzapine-treated compared to unmedicated patients. Transcriptional activation of lipid biosynthesis genes in peripheral blood cells of olanzapine-treated patients suggests a direct lipogenic action of antipsychotic drugs, which may be related to metabolic adverse effects.

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Introduction
Treatment with antipsychotic drugs has been associated with weight gain, dyslipidaemia and type 2 diabetes (Consensus Statement, 2004). Such metabolic adverse effects may increase the risk of cardiovascular disease, which is already a major clinical problem in patients with severe mental disorders (Brown, 1997; Marder et al., 2004; Osborn et al., 2007). However, several findings indicate that different antipsychotics carry varying amounts of risk. Clozapine and olanzapine are the ones associated with the highest level of metabolic disturbances (Allison and Casey, 2001; Lamberti et al., 2006; Nasrallah, 2006; Smith et al., 2005). The underlying molecular pathways for these side-effects are not yet established, although a CNS-mediated orexigenic action of antipsychotics has recently been shown (Kim et al., 2007; Kroeze et al., 2003).

One possible peripheral mechanism for drug-induced metabolic adverse effects, such as dyslipidaemia, might be enhanced lipogenesis through increased enzymatic activity. Fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) are two central enzymes in fatty-acid biosynthesis. FASN catalyses de-novo synthesis of fatty acids, while SCD converts palmitoyl-CoA and stearoyl-CoA to oleate and palmitoleate, which are the most abundant mono-unsaturated fatty acids in membrane phospholipids, triglycerides and cholesterol esters. In healthy human subjects, expression of the FASN gene in leukocytes has been shown to correlate with blood lipid levels.
Elevated expression of SCD in skeletal muscle contributes to abnormal lipid metabolism in obese humans (Hulver et al., 2005), and Scd1-deficient mice display reduced lipid synthesis and are resistant to diet-induced weight gain (Ntambi et al., 2002).

We have recently shown that antipsychotics in general, and clozapine in particular, increase cellular lipogenesis in several different human cell cultures, including both glial and liver cells (Ferno et al., 2005, 2006; Raeder et al., 2006). This effect is mediated by drug-induced activation of a key transcriptional factor, sterol regulatory element-binding protein (SREBP), with subsequent up-regulation of downstream genes involved in cholesterol (e.g. HMGCS1 and HMGCR) and fatty acid (e.g. FASN and SCD) biosynthesis. Interestingly, Minet-Ringuet et al. (2007) recently demonstrated that FASN was up-regulated in adipocytes isolated from rats exposed to olanzapine for 5 wk. These findings suggest SREBP activation as a possible mechanism in the development of antipsychotic-induced metabolic adverse effects. The aim of the present study was to explore this hypothesis by investigating the gene expression of FASN and SCD in whole blood of patients receiving olanzapine in monotherapy, compared to unmedicated patients.

Methods

The ongoing TOP (Thematic Organized Psychosis Research) Study is carried out in joint collaboration between the University of Oslo and university hospitals in Oslo, Norway. The study design is naturalistic, and inclusion criteria are broad, consisting of (1) being registered in the psychiatric services of any one of the four university hospitals in Oslo, (2) aged 18–65 yr, and (3) meeting DSM-IV criteria for a major psychotic illness. The methods of the TOP Study, along with the demographic and clinical features of the first 205 patients, are described in greater detail elsewhere (Birknaes et al., 2006). The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All subjects gave informed, written consent for participation after having received a detailed description of the study.

A subsample of 48 patients was consecutively recruited into one of two groups according to the following criteria: Medicated subjects [continuous exclusive monotherapy with olanzapine for ≥3 wk prior to testing (n = 19)], or Unmedicated subjects [no use of pharmacological agents in the last 3 wk prior to testing (n = 29)]. The groups were then matched, based on gender, race and body mass index (BMI), all of which are known to be of importance for lipid levels. The matching procedure was performed blindly to the gene expression data, leaving 38 patients (19 in each group) accessible for statistical analyses. Individuals with drug abuse were excluded from both study groups.

The medicated group (n = 19) received daily doses of olanzapine ranging from 2.5 to 15 mg, with a mean (s.d.) dose of 8.7 (3.5) mg. Median duration of olanzapine treatment was 12 months (range 1–90 months). Measurement of serum olanzapine concentration was available for 15 patients, with a median level of 37.0 nmol/l (range 8–142 nmol/l); recommended therapeutic level in Norway is 30–200 nmol/l. In the unmedicated group (n = 19), eight subjects were naive to antipsychotic medication, six had previously used antipsychotic drugs other than clozapine or olanzapine, while five had previously been on olanzapine, but only two of these for a period of >2 wk. None of the unmedicated patients had used antipsychotics for the last 2 months.

Clinical examinations were performed and blood samples drawn from all patients in the morning after an overnight fast of at least 8 h. The Paxgene RNA blood system (PreAnalytiX; Qiagen, Hilden, Germany and Becton Dickinson and Company, Franklin Lakes, NJ, USA) was used for collecting, stabilizing and purifying total RNA prior to gene expression analysis. We only examined the FASN and SCD genes, and this selection was based on their important function in fatty-acid biosynthesis and data from recent studies in our laboratory of antipsychotic-exposed cell cultures (Ferno et al., 2005, 2006; Raeder et al., 2006). Relative expression levels of SCD and FASN were determined at the Centre for Medical Genetics and Molecular Medicine, Haukeland University Hospital, University of Bergen, using a quantitative real-time polymerase chain reaction with the fluorescent marker SybrGreen for target genes and TaqMan-based probes for the endogenous control (see Ferno et al., 2006 for further details). Ribosomal protein P0 was chosen as the endogenous control for normalization; 18S and β-actin both gave qualitatively similar results (data not shown). Samples were quantified using the standard curve method, and expression was denoted as percentage compared to mean normalized expression levels of the control group.

Analyses of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were performed at the Department of Clinical Chemistry, Ulleval University Hospital, on an Integra 800 (Roche Diagnostics, Mannheim, Germany), using standard methods. Serum concentrations of olanzapine were determined at the
Department of Clinical Pharmacology, St Olav’s Hospital, Trondheim.

**Statistical procedures**

All statistical analyses were performed using the SPSS software package for Windows version 14.00 (SPSS, Chicago, IL, USA). Background variables and gene expression data are presented as mean values or proportions. In comparisons between groups, we used Student’s *t* tests for continuous variables, and Pearson’s *χ*² tests for categorical variables. A univariate analysis of covariance was used in order to adjust for inter-group age differences on lipid levels. Data are presented as mean adjusted values (95% confidence interval). In general, two-sided tests were used, and the significance level was set at 0.05.

**Results**

Table 1 shows background data as well as lipid and gene expression levels for medicated vs. unmedicated patients. The medicated group was somewhat younger, and consisted of more subjects with schizophrenia and more non-smokers. Duration of illness was highly similar between groups. In line with the BMI matching, lipid levels were not statistically different between the two groups.

Both target genes were significantly up-regulated in whole blood obtained from the olanzapine-treated group. *FASN* was increased 1.53-fold (*p* = 0.003) and *SCD* was enhanced 1.62-fold (*p* = 0.002), compared to the unmedicated group. For one medicated subject, we also had gene expression data prior to initiation of olanzapine treatment (not included in the analysis). For this patient, the relative expression of *FASN* increased from 0.95 to 1.47, and expression of *SCD* increased from 1.47 to 2.80 within 1 month of olanzapine treatment (data not shown). There was no significant association between smoking and gene expression levels (data not shown). To control for the matching procedure, we also examined and compared the gene expression levels for all olanzapine-treated (*n* = 19) and unmedicated (*n* = 29) subjects included in the study, showing that both *FASN* (*p* = 0.006, d.f. = 23.60) and *SCD* (*p* = 0.008, d.f. = 23.52) were still significantly up-regulated in the olanzapine group (unequal variances assumed; data not shown).

The limited number of participants in the study markedly reduces the power of correlation analysis. However, we still observed a significant positive correlation between *FASN* and *SCD* expression levels in whole blood obtained from the medicated group.

### Table 1. Gene expression in olanzapine-medicated vs. unmedicated patients

<table>
<thead>
<tr>
<th></th>
<th>Medicated (n = 19)</th>
<th>Unmedicated (n = 19)</th>
<th>Levene’s <em>F</em> value</th>
<th>Pearson <em>r</em> value</th>
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<tbody>
<tr>
<td><strong>Background variables</strong></td>
<td></td>
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<tr>
<td>Male, % (n)</td>
<td>68.4 (13)</td>
<td>68.4 (13)</td>
<td>-a</td>
<td></td>
</tr>
<tr>
<td>White, % (n)</td>
<td>84.2 (16)</td>
<td>89.5 (17)</td>
<td>-a</td>
<td></td>
</tr>
<tr>
<td>Age (yr), mean (S.D.)</td>
<td>33.6 (12.0)</td>
<td>38.4 (13.4)</td>
<td>0.61</td>
<td>36</td>
</tr>
<tr>
<td>Schizophrenia, % (n)</td>
<td>84.2 (16)</td>
<td>63.2 (12)</td>
<td>2.17</td>
<td>1</td>
</tr>
<tr>
<td>Illness duration (yr), mean (S.D.)</td>
<td>6.4 (6.3)</td>
<td>6.2 (6.9)</td>
<td>0.22</td>
<td>36</td>
</tr>
<tr>
<td>Smokers, % (n)</td>
<td>31.6 (6)</td>
<td>63.2 (12)</td>
<td>3.80</td>
<td>1</td>
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<tr>
<td><strong>Metabolic variables,</strong></td>
<td></td>
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<tr>
<td>age adjusted means (95% CI)</td>
<td></td>
<td></td>
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<tr>
<td>Body mass index</td>
<td>25.3 (23.2–27.3)</td>
<td>25.2 (23.3, 27.2)</td>
<td>-a</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.3 (4.9–5.7)</td>
<td>5.4 (5.1, 5.8)</td>
<td>0.26</td>
<td>1</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.3 (3.0–3.6)</td>
<td>3.5 (3.2, 3.8)</td>
<td>0.44</td>
<td>1</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.3 (1.1–1.5)</td>
<td>1.4 (1.2, 1.6)</td>
<td>0.79</td>
<td>1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.5 (1.2–1.9)</td>
<td>1.2 (0.8, 1.5)</td>
<td>1.89</td>
<td>1</td>
</tr>
<tr>
<td><strong>Gene expression in whole blood,</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>mean values (S.D.)</td>
<td></td>
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</tr>
<tr>
<td><em>FASN</em> expression</td>
<td>1.53 (0.60)</td>
<td>1.00 (0.38)</td>
<td>4.58</td>
<td>30.41b</td>
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<tr>
<td><em>SCD</em> expression</td>
<td>1.62 (0.73)</td>
<td>1.00 (0.27)</td>
<td>20.53</td>
<td>22.84b</td>
</tr>
</tbody>
</table>

LDL, Low-density lipoprotein; HDL, high-density lipoprotein.

*a* The two groups were matched, based on gender, race and body mass index.

*b* Unequal variances assumed.
olanzapine-medicating patients ($p=0.012$, Spearman’s rank, two-sided, $n=19$), while there was no such correlation in the unmedicated patients ($p=0.273$, $n=19$) (data not shown). Moreover, there was no significant correlation between drug concentrations or duration of treatment vs. gene expression levels.

**Discussion**

The main finding of the present study was a significantly increased expression of two central lipid biosynthesis genes in peripheral blood cells of olanzapine-treated compared to unmedicated patients. To our knowledge, this is the first report of antipsychotic-induced enhanced expression of FASN and SCD genes in patients.

Numerous studies have shown that treatment with antipsychotic drugs, in particular clozapine and olanzapine, is associated with weight gain, insulin resistance and dyslipidaemia, and the risk of drug-induced metabolic disturbances has become a focus of great clinical concern (Allison and Casey, 2001; Henderson, 2005; Marder et al., 2004). Studies addressing the question of whether such adverse effects are mediated via the CNS or peripheral tissue action have been requested (Consensus Statement, 2004). An influence of antipsychotic drugs on the brain, especially the hypothalamus, may affect the regulation of appetite and energy expenditure. In line with this hypothesis, it has been reported that the affinity of both typical and atypical antipsychotics to histamine $H_1$ and serotonin 5-HT$_2C$ receptors is correlated with the risk of drug-induced weight gain and diabetes mellitus (Kroeze et al., 2003; Matsui-Sakata et al., 2005). Moreover, it has been shown that weight gain-inducing antipsychotics (clozapine and olanzapine) but not weight neutral drugs (ziprasidone) potently stimulate the orexigenic hypothalamic AMP-kinase through histamine $H_1$ receptors (Kim et al., 2007).

Still, several lines of evidence indicate that mechanisms outside of the CNS are also involved. Metabolomic mapping of global lipid changes in patients with schizophrenia has demonstrated that olanzapine affects a much broader range of lipid classes than aripiprazole, with increased concentrations of triglycerides (Kaddurah-Daouk et al., 2007). We have shown that antipsychotic drugs activate SREBP transcription factors in cultured human glioma and liver cells, with consequent up-regulation of downstream lipogenic gene expression and increased biosynthesis of cholesterol and triglycerides (Ferno et al., 2005, 2006; Raeder et al., 2006). Clozapine and chlorpromazine were the most potent drugs in a context of therapeutically relevant concentrations, whereas ziprazidone induced minimal or no SREBP activation (Ferno et al., 2006). Our present findings of similarly increased expression of the SREBP-controlled FASN and SCD genes in whole blood of olanzapine-treated patients indicate that antipsychotics also activate the SREBP transcription system in peripheral blood cells in vivo. Interestingly, the expression level of FASN and SCD was positively correlated in the group of olanzapine-medicating patients but not in the unmedicated subjects, further supporting the transcriptional activation of the olanzapine treatment on fatty-acid biosynthesis. The current cross-sectional study, with BMI-matched groups, was not designed to detect differences in lipid parameters and there was no significant correlation between gene expression and lipid levels. However, other studies have shown that blood lipid levels in healthy human subjects were correlated with SREBP-regulated gene expression in leukocytes (Ma et al., 2006). We therefore suggest SREBP activation as a potential mechanism of antipsychotic action in peripheral tissues, such as liver and adipocytes, leading to dyslipidaemia.

There was an almost perfect match between the study groups on important background variables such as gender, race and BMI, strengthening the conclusion that the observed difference in gene expression is due to the olanzapine treatment and not any confounding factor. A non-significant difference in the distribution of diagnoses between the two groups was considered to be of less importance, especially since we have previously shown that in the TOP Study sample as a whole, patients with schizophrenia and affective disorders had the same level of metabolic risk parameters (Birkenaes et al., 2007). Smoking was less prevalent in the olanzapine-treated group, but since smoking in general is pro-dyslipidaemic (Craig et al., 1989), the over-representation of smokers among the unmedicated subjects should, if anything, have attenuated the difference in gene expression between the two groups. In addition, there was no significant association between smoking and gene expression.

However, the study has methodological weaknesses, including a cross-sectional design, relative small sample sizes and unmedicated control subjects who were not all naive to previous antipsychotic drug treatment. In the latter patients, previous exposure to olanzapine or related orexigenic antipsychotics may have brought about changes in lipid homeostasis and body composition with long-lasting effects, but this potential bias was only relevant for a few patients...
and should have masked (rather than increased) the observed difference in gene expression levels.

In conclusion, we propose that antipsychotic-induced activation of SREBP-controlled lipid biosynthesis could be an alternative mechanism for drug-related lipogenesis in peripheral tissues, linked to metabolic adverse effects such as dyslipidaemia. This hypothesis should be further tested in a larger, prospective sample, ideally with drug-naive subjects.

Acknowledgements

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Statement of Interest

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