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Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro

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ABSTRACT

Schizophrenia requires lifelong treatment, potentially causing systemic changes in metabolic homeostasis. In the clinical setting, antipsychotic treatment may differentially lead to weight gain among individual patients, although the molecular determinants of such adverse effects are currently unknown. In this study, we investigated changes in the expression levels of critical regulatory genes of adipogenesis, lipid metabolism and proinflammatory genes during the differentiation of primary human adipose-derived stem cells (ADSCs). These cells were isolated from patients with body mass indices <25 and treated with the second-generation antipsychotics olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole and risperidone and the first-generation antipsychotic haloperidol. We found that antipsychotics exhibited a marked effect on key genes involved in the regulation of cell cycle, signal transduction, transcription factors, nuclear receptors, differentiation markers and metabolic enzymes. In particular, we observed an induction of the transcription factor NF-KB1 and NF-KB1 target genes in adipocytes in response to these drugs, including the proinflammatory cytokines TNF-α, IL-1β, IL-8 and MCP-1. In addition, enhanced secretion of both IL8 and MCP-1 was observed in the supernatant of these cell cultures.

In addition to their remarkable stimulatory effects on proinflammatory gene transcription, three of the most frequently prescribed antipsychotic drugs, clozapine, quetiapine and aripiprazole, also induced the expression of essential adipocyte differentiation genes and the adipocyte hormones leptin and adiponectin, suggesting that both glucose and fat metabolism may be affected by these drugs. These data further suggest that antipsychotic treatments in patients alter the gene expression patterns in adipocytes in a coordinated fashion and priming them for a low-level inflammatory state.

1. Introduction

Atypical antipsychotics (AAPs), or second-generation antipsychotics (SGAs), are widely prescribed for the treatment of several psychiatric disorders. However, these drugs are associated with many mild and serious side effects. The major side effects of AAPs are weight gain and its associated metabolic disorders, such as type II diabetes and dyslipidemia [1,2]. The increase in obesity-related adipose tissue mass may derive from both increased adipocyte size due to lipid accumulation in differentiated adipocytes, and increased adipocyte number due to the differentiation of adipose-derived stem cells (ADSCs) present in adipose tissue [3]. Studies in cultured rodent adipocytes suggest that certain AAPs can facilitate lipid storage and stimulate adipogenesis [4,5]. However, there is limited information regarding the effect of AAPs on human preadipocytes [6]. It is not yet known how AAPs affect the differentiation process of resident preadipocytes or the terminally differentiated adipocytes, or whether increased lipid storage could cause a level of cellular stress high enough to trigger a cell death pathway at the gene expression level in adipocytes. Nevertheless, obesity is often associated with a low-grade state of inflammation that is attributed to the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, and monocyte chemoattractant protein-1 (MCP-1) in adipose tissues. As a consequence of this inflammatory environment, macrophages are recruited to the adipose tissue and, in turn, produce additional inflammatory mediators [7]. The aim of the

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Table 1
Biochemical classification of the studied genes.

Studied genes		
Cell cycle	ANAPC2	Anaphase promoting complex subunit 2
	CDK4	Cyclin-dependent kinase 4
Apoptosis	BCL2	B-cell leukemia/lymphoma 2
	BAX	B-cell leukemia/lymphoma 2
Receptors and transporters	ABCA1	ATP-binding cassette, sub-family A member 1
	LEPR	Leptin receptor
	INSR	Insulin receptor
Signal transduction	GHR	Growth hormone receptor
	IRS1	Insulin receptor substrate 1
	PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
	SIRT1	Sirtuin 1
Transcription factors	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
	SREBF1	Sterol regulatory element binding transcription factor 1
	NFκB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
Nuclear receptors	PPARA	Peroxisome proliferator activated receptor alpha
	PPARG	Peroxisome proliferator-activated receptor gamma
Adipogenic differentiation markers	ADFP	Adipose differentiation related protein
	FABPN	Fatty acid bindin protein
Lipid metabolism enzymes	LPL	Lipoprotein lipase
	ACSL1	Acyl-CoA synthetase long-chain family member 1
Adipokines	ADIPOQ	Adiponectin
	LEP	Leptin
Cytokines and chemokines	TNF	Tumor necrosis factor
	CCL2	Chemokine (C-C motif) ligand 2
	IL1β	Interleukin 1, beta
	IL8	Interleukin 8

The expression of 26 genes in adipocytes treated with antipsychotics during adipogenic differentiation were measured using qPCR.

present study was to investigate the effect of AAP treatment on differentiated adipocytes. For this purpose, we designed a qPCR array to investigate changes in gene expression in differentiated adipocytes in the presence of AAPs. The PCR array measured the expression of 26 genes (Table 1) encoding important regulators of adipocyte function, signaling molecules involved in energy storage and expenditure, and those related to obesity. For these expression studies, we used six SGAs (olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole and risperidone) and one first-generation antipsychotic (haloperidol). To date, only a few studies have examined the effect of the AAP drugs used for the treatment of psychiatric disorders at the gene expression level [5,6,8–10].

In this study we found a concerted induction of proinflammatory genes and upregulation of inflammatory mediators in response to a versatile group of antipsychotic drugs. Three of the most potent agents, clozapine, quetiapine and aripiprazole, demonstrated a clear propensity to also induce adipogenic genes.

2. Material and methods

2.1. Selection of preadipocyte tissue donors

Preadipocytes were obtained from subcutaneous abdominal adipose tissue of four healthy males aged 45–75 years who underwent a planned surgical treatment (herniotomy). The study protocol was approved by the Ethics Committee of the University of Debrecen, Hungary (No. 3186-2010/DEOEC RKEB/IKEB).

2.2. Preadipocytes were isolated, cultured and differentiated as described previously [11]

2.2.1. Drug treatment

The seven schizophrenia drugs were dissolved in DMSO (Sigma) and used in the following final concentrations: olanzapine 50 ng/mL, ziprasidone 50 ng/mL, clozapine 100 ng/mL, quetiapine 50 ng/mL, aripiprazole 100 ng/mL, haloperidol 10 ng/mL, risperidone 50 ng/mL. Drugs were added on the first day of differentiation of adipocytes and then subsequently every day until day 11. The cell culture media were replaced every third day.

2.3. PCR array

mRNA expressions were determined with CAPH09329 Custom Human RT2 ProfilerTM PCR Arrays (SABiosciences). cDNA synthesis, labeling and hybridization were carried out according to manufacturer's protocol. The fold changes for target genes presented in the figures and Supplementary information were calculated as the ratio of expression levels of the (untreated) control and AAP-treated differentiated adipocytes.

To determine which changes in gene expression were most closely correlated, the fold-changes of relative expression levels were log2-transformed and clustered by complete linkage of Euclidian distances using the Gene Cluster 3.0 software and visualized on heat maps using TreeView (Eisen lab, UC Berkeley).

2.4. Determination of cytokine release

Culture supernatants were harvested during drug treatment and stored for cytokine measurements. Media from the same donor and drug-treated sample were pooled, and the level of IL-8 and MCP-1 was measured using an ELISA DuoSet Kit (R&D). Assays were performed according to the manufacturer's protocols.

For the statistical analyses, a two-tailed paired t-test was applied. $p < 0.05$ was considered statistically significant.