Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1884–1890

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Effects of antipsychotics with different weight gain liabilities on human *in vitro* models of adipose tissue differentiation and metabolism

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ARTICLE INFO

Article history:
Received 19 April 2011
Received in revised form 11 July 2011
Accepted 28 July 2011
Available online 5 August 2011

Keywords:
Adipocyte-derived stem cells (ADSCs)
Antipsychotics
Human adipose tissue
Mature adipocytes
Metabolic abnormalities
Weight gain

ABSTRACT

Weight gain and metabolic abnormalities are serious side effects associated with the use of several second generation antipsychotics (SGA). The adipose tissue has been considered a direct SGA target involved in the development of these adverse effects. Recent studies, mainly using murine cells, have suggested that SGA increase both adipogenesis of preadipocytes and lipid accumulation in mature adipocytes. However, to date there has been little research comparing the effects of antipsychotics with different propensities to induce weight gain on human *in vitro* models of white adipose tissue neoformation and metabolism. The present study aimed to investigate the effects of antipsychotics either strongly associated with weight gain, such as the SGA clozapine and olanzapine, or not, such as the SGA ziprasidone and the classical antipsychotic haloperidol, on proliferation and adipocyte differentiation of human adipose-derived stem cells (ADSCs) and lipogenesis in human mature adipocytes. Whereas ziprasidone induced elevated levels of cell death during adipogenesis and could not be investigated further, we observed that clozapine, olanzapine and haloperidol had slight stimulatory effects on the transcriptional program of ADSCs adipogenesis. However, the observed changes in adipocyte-specific genes were not accompanied by a significant increase in triglyceride accumulation within differentiated adipocytes. Our data also showed that these three antipsychotics displayed inhibitory effects on the proliferation rates of undifferentiated ADSCs. Regarding mature adipocyte metabolism, we observed that olanzapine slightly inhibited insulin-stimulated lipogenesis at the highest concentration used, and haloperidol exerted the strongest inhibitory effects on both basal and insulin-stimulated lipogenesis. Taken together, our results suggest that a direct and potent effect of clozapine and olanzapine on adipose tissue biology is not an important mechanism by which these SGA induce metabolic disturbances in humans. On the other hand, the haloperidol-mediated downregulation of the lipogenic capacity of human adipose tissue may be a possible mechanism contributing to its lower propensity to induce serious metabolic side effects.

1. Introduction

Second generation antipsychotic drugs (SGA) are considered to possess superior efficacy in treating both positive and negative symptoms of schizophrenia compared to first-generation antipsychotics (FGA) (e.g. haloperidol) (Bridler and Umbricht, 2003; Keefe et al., 2006). In addition, these drugs are increasingly being prescribed for other psychiatric disorders, such as bipolar disorder, autism, and depression (McDougle et al., 2008; Papakostas and Shelton, 2008; Pfeifer et al., 2010). However, major side effects of SGA, such as clozapine and olanzapine, are weight gain and associated metabolic abnormalities including hyperglycemia, insulin resistance, type II diabetes, dyslipidemias and cardiovascular diseases (Ananth and Kolli, 2005; Casey, 2005; Newcomer, 2007; Panagiotopoulos et al., 2009).

Abbreviations: ADSCs, human adipose-derived stem cells; BMI, body mass index; *C/EBPβ*, CCAAT/enhancer-binding protein β; Ct, cycle threshold; Cy5, cyanine 5; DDX58, DEAD-box protein 58; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FGA, first-generation antipsychotics; FITC, fluorescein isothiocyanate; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *HMBS*, hydroxymethylbilane synthase; *HPRT1*, hypoxanthine phosphoribosyltransferase 1; IBMX, 3-isobutyl-1-methylxanthine; *LPL*, lipoprotein lipase; NF, normalization factor; NP-40, nonidet P-40; OD, optical density; PBS, phosphate-buffered saline; PE, phycoerythrin; *PPARγ2*, peroxisome proliferator-activated receptor γ2; SD, standard deviation; *SDHA*, succinate dehydrogenase complex subunit A; SGA, second generation antipsychotics; TG, triglyceride; XTT, tetrazolium salt 3′-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate.

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doi:10.1016/j.pnpbp.2011.07.017

Although the exact mechanisms involved in SGA-induced weight gain and metabolic abnormalities are not yet fully understood, several studies have proposed that these side effects may reflect the antagonist properties of these drugs on central nervous system pathways involved in the food intake or energy expenditure, such as histaminergic, serotoninergic/noradrenergic pathways (Deng et al., 2009; Han et al., 2008; Matsui-Sakata et al., 2005; Opgen-Rhein et al., 2010; Reynolds et al., 2002). Other studies have also explored a direct effect of some SGA on the biology of peripheral tissues involved in body energy homeostasis, such as the adipose tissue.

Increased white adipose tissue mass associated with obesity may be the function of both, increased adipocyte size, due to lipid accumulation within fully differentiated adipocytes, and increased adipocyte number, due to proliferation and adipogenic differentiation of progenitor cells present in the adipose tissue (Spalding et al., 2008). Recent reports have suggested that specific SGA can exert direct effects on mature rat adipocytes from visceral white adipose tissue, increasing lipogenesis and decreasing lipolysis to facilitate adipocyte lipid storage (Minet-Ringuet et al., 2007; Vestri et al., 2006). In addition, it was proposed that the SGA clozapine and olanzapine stimulate adipogenesis of the murine preadipocyte cell line 3T3L1 (Yang et al., 2007; 2009). However, there are very limited data concerning the effects of SGA on cells derived from human adipose tissue (Hemmrich et al., 2006; Pavan et al., 2009), remaining substantial uncertainties over these effects.

The present study aimed to extend the findings of prior studies and compared the effects of antipsychotics with different weight gain liabilities, such as the SGA clozapine and olanzapine and the FGA haloperidol, on proliferation and adipocyte differentiation of human adipose-derived stem cells (ADSCs), as well as lipogenesis in human mature adipocytes.

2. Material and methods

2.1. Adipocyte-derived stem cells (ADSCs) isolation and expansion

Liposuction aspirates from abdominal subcutaneous adipose tissue were obtained from 5 health female subjects (aged 24–38 years; BMI<25 kg/m^2) undergoing cosmetic surgery procedures performed under general anesthesia (Keck et al., 2010). In order to limit variables or confounding factors which might affect adipose tissue function, subjects were devoid of metabolic, endocrine, or cardiovascular diseases. The study was approved by local ethics committee, and patients gave their informed consent for using their ADSCs for the present experiments. ADSCs were isolated by collagenase digestion (0.075% collagenase in PBS; Sigma type IA collagenase) as described previously (Zuk et al., 2001), counted using the Trypan blue dye exclusion assay, and then seeded on plastic tissue culture plates at a density of 4000 cell/cm^2 in DMEM-low glucose medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen/Gibco) and antibiotics (defined as ADSCs growth medium). After achieving a density of 80–90%, cultures were trypsinized and subcultured at a 1:4 ratio. All tests were performed at passage 4.

2.2. Immunophenotyping

ADSC populations used in the experiments were analysed with specific cell surface markers to evaluate homogeneity. Cells were harvested with trypsin, centrifuged at 500 *g* for 5 min, washed with phosphate-buffered saline (PBS), and resuspended in PBS. Cells (1×10^5 cells/tube) were then incubated at 4 °C for 30 min with the following antibodies: CD29-PE-Cy5, CD90 (Thy-1)-PE-Cy5, CD45-FITC, CD31-PE, CD105 (SH2) and CD73 (SH3/4) (Becton Dickinson). After a second wash with PBS, samples incubated with unconjugated primary antibodies were incubated with anti-mouse-PE secondary antibody (Guava Technologies) for additional 15 min at 4 °C. Finally, the cell suspension was washed with PBS, and 10^5 labeled cells were acquired with an EasyCyte Flow cytometer (Guava Technologies). Control samples were incubated with PBS instead of primary antibody, followed by incubation with anti-mouse-PE secondary antibody. All the plots generated were analysed with Guava ExpressPlus software (Guava Technologies).

2.3. Adipocyte differentiation and pharmacological treatment

To induce adipocyte differentiation, ADSCs were grown to confluence (defined as day 0) and then cultured in adipogenic induction medium [DMEM high glucose (25 mM) supplemented with 10% FBS, antibiotics, 1 μM dexamethasone (Sigma), 200 μM indomethacin (Sigma), 0.5 mM IBMX (Sigma), and 10 μg/ml insulin (Sigma)] for 14 days, which represents the usual time needed to acquire a fully differentiated phenotype. The influence of the different antipsychotic drugs on adipogenesis was tested by supplementing the differentiation medium with clozapine (Sigma, MO, USA), olanzapine (Toronto Research Chemicals, Toronto, Canada), ziprasodone (Pfizer inc., NY, USA) or haloperidol (Sigma, MO, USA) at different concentrations during the first stage of differentiation (either for the first 3 days or for the first 7 days during differentiation). The antipsychotic drugs were used at doses similar to their therapeutic plasma concentrations [considered to be in the range of 0.6 to 1 μM clozapine (Broich et al., 1998); 0.07–0.2 μM olanzapine (Bergemann et al., 2004; Robinson et al., 2006); 0.1–0.25 μM ziprasidone (Vogel et al., 2009); and 0.05 μM haloperidol (Coryell et al., 1998)], at doses 10-fold higher than those (10 μM clozapine; 2 μM olanzapine; 2.5 μM ziprasidone; 0.5 μM haloperidol), or at 20, 30, 40 and 100 μM. Control cells were treated with DMSO alone (solvent used to dissolve the drugs) and no effect of this solvent on adipogenesis was observed (data not shown).

2.4. Cell viability analysis during adipogenic differentiation

Cells were plated into 12-well cell culture plates (1.5×10^4 cells/well) and grown to confluence (day 0). The cells were then cultured in adipogenic induction medium supplemented with DMSO (control) or clozapine, olanzapine, ziprasidone or haloperidol at different concentrations, as described above. On day 14 after induction, the cells were trypsinized, stained with trypan blue and counted with an Automated Cell Counter (Invitrogen). The experiment was done in triplicates for each antipsychotic treatment and each cell line. The data were normalized to cell number of DMSO-treated samples.

2.5. RNA extraction, reverse transcription reactions and quantitative real time PCR

Cells for expression analysis were grown in 75 cm^2 plastic culture flasks. Total RNA from confluent undifferentiated ADSCs (day 0), and from cells cultured in adipogenic induction medium supplemented or not (DMSO alone) with the antipsychotics was isolated using Nucleospin RNA kit (Macherey-Nagel). RNA quality and concentration were accessed respectively by 1.5% agarose gel electrophoresis and Nanodrop ND-1000. Complementary DNA (cDNA) was produced from 1.5 μg of total RNA using Superscript II reverse transcription kit (Invitrogen). Quantitative real-time PCR was performed using approximately 15 ng of cDNA and SYBR Green PCR master mix in an ABI Prism 7500 system (Applied Biosystems). Primers were designed using Primer Express software v2.0 (Applied Biosystems) and the amplification efficiency (E) of each primer was calculated according to the equation $E = 10^{(-1/\text{slope})}$. The expression data of the adipocyte-specific transcripts *C/EBPβ*, *PPARγ2* and *LPL*, as well as an unrelated transcript *DDX58*, were determined by relative quantification in comparison to four endogenous controls *HMBS*, *HPRT1*, *GAPDH* and *SDHA*. GeNorm v3.4 was used to determine the most stable controls