Progress in Neuro-Psychopharmacology & Biological Psychiatry 48 (2014) 170–176

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Antidiabetic-drug combination treatment for glucose intolerance in adult female rats treated acutely with olanzapine

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ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 23 September 2013
Accepted 7 October 2013
Available online 16 October 2013

Keywords:
Animal model
Glyburide
Insulin resistance
Metformin
Olanzapine
Rosiglitazone

ABSTRACT

Second generation antipsychotic drugs are routinely used as treatment for psychotic disorders. Many of these compounds, including olanzapine, cause metabolic side-effects such as impaired glucose tolerance and insulin resistance. Individual antidiabetic drugs can help control elevated glucose levels in patients treated with antipsychotics, but the effects of combining antidiabetics, which routinely occurs with Type 2 diabetes mellitus patients, have never been studied. Presently, we compared the effects of the three different antidiabetics metformin (500 mg/kg, p.o.), rosiglitazone (30 mg/kg, p.o.) and glyburide (10 mg/kg, p.o.) on metabolic dysregulation in adult female rats treated acutely with olanzapine. In addition, dual combinations of each of these antidiabetics were compared head-to-head against each other and the individual drugs. The animals received two daily treatments with antidiabetics and were then treated acutely with olanzapine (10 mg/kg, i.p.). Fasting glucose and insulin levels were measured, followed by a 2 h glucose tolerance test. Olanzapine caused a large and highly significant glucose intolerance compared to vehicle treated rats. Rosiglitazone decreased glucose levels non-significantly, while both metformin and glyburide significantly decreased glucose levels compared to olanzapine-only treated animals. For antidiabetic dual-drug combinations, the rosiglitazone–metformin group showed an unexpected increase in glucose levels compared to all of the single antidiabetic drugs. However, both the metformin–glyburide and rosiglitazone–glyburide groups showed significantly greater reductions in glucose levels following olanzapine than with single drug treatment alone for metformin or rosiglitazone, bringing glucose levels down to values equivalent to vehicle-only treated animals. These findings indicate that further study of antidiabetic dual-drug combinations in patients treated with antipsychotic drugs is warranted.

1. Introduction

The second generation (atypical) antipsychotic drugs represent the first line of pharmacotherapy treatment for schizophrenia spectrum disorders (Honer et al., 2007). Second generation antipsychotic drugs are also increasingly being used for additional psychiatric indications, such as bipolar disorder, major depression and anxiety disorders (Maher et al., 2011; Procyshyn et al., 2010). The use of second generation antipsychotics in some populations, such as adolescents, has increased dramatically over the past decade (Patten et al., 2012). This is, in large part, because most second generation antipsychotics offer the advantage of a low incidence of motor side-effects (Nasrallah, 2006), as well as a decreased incidence of other sequelae associated with the first generation drugs, such as hyperprolactinemia.

It is now well-established, however, that many of the second generation antipsychotic drugs are associated with harmful metabolic side-effects (Henderson, 2007; Newcomer, 2007; Procyshyn et al., 2007). Converging evidence from many clinical studies has demonstrated that treatment with second generation antipsychotics can cause weight gain, hyperlipidemia, hyperglycemia and insulin resistance, resulting in metabolic syndrome and ultimately causing Type 2 diabetes mellitus (DM) (Newcomer, 2005; Reynolds, 2007), with its associated cardiovascular complications (Leung et al., 2012).

General awareness of the harmful metabolic side-effects of second generation antipsychotic drugs has increased in recent years (Papanastasiou, 2012), but options available to prevent these adverse events are limited. Moderate success in controlling metabolic symptoms has been achieved using lifestyle changes, which include regular exercise and improvement of diet (Park et al., 2011). Adherence to these types of volitional interventions, however, may be even more of

Abbreviations: AUC, Area under the curve; i.p., Intraperitoneal; LSD, Least significant difference; NIH, National Institutes of Health; PO, Per os; PPAR, Peroxisome proliferator-activated receptor; RPM, Revolutions per minute; t, Time; UBC, University of British Columbia.

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http://dx.doi.org/10.1016/j.pnpbp.2013.10.006

a challenge in psychiatric patients than in the general population (von Hausswolff-Juhlin et al., 2009). Thus, the use of antidiabetic drugs represents a key clinical option for many patients treated with antipsychotic drugs who suffer from obesity, hyperglycemia and insulin resistance. Modest beneficial effects have been observed on multiple metabolic indices in patients treated with second generation antipsychotics using various antidiabetic drugs (Baptista et al., 2008a, 2009; Carrizo et al., 2009; Maayan et al., 2010; Tse et al., in press). However, to our knowledge, there have been no studies – either in animals or in humans – of the effects of antidiabetic drug combination therapy in patients treated with antipsychotic drugs. In the general population, different classes of antidiabetic drugs are commonly co-prescribed when individual antidiabetic drugs do not provide adequate control over metabolic symptoms (Goldman-Levine, 2011). This strategy works, in large part, because the different classes of antidiabetic drugs work through independent biochemical pathways, and are thus able to create additive effects.

Preclinical models of antipsychotic drug-induced metabolic side effects have demonstrated good predictive validity (Boyda et al., 2010a). When compared against each other, the antipsychotic drugs that have the most severe metabolic side-effects in rats typically correspond to those with the greatest metabolic liability in humans (Boyda et al., 2010b; Chintoh et al., 2009; Smith et al., 2008). A handful of preclinical studies have also shown that the metabolic side-effects of antipsychotics can be ameliorated by exercise (Boyda et al., in press) or treatment with antidiabetic drugs (Adeneye et al., 2011; Arulmozhi et al., 2006; Lykkegaard et al., 2008; Smith et al., 2009). Recently, we demonstrated that the antidiabetic drugs metformin and rosiglitazone, but not glyburide, could significantly reverse the hyperglycemia caused by olanzapine (Boyda et al., 2012a). The goal of the present study was therefore to use these same drugs and determine whether combining the antidiabetics, as occurs with Type 2 DM, could provide additional metabolic benefits compared to treatment with the single antidiabetic drug alone.

2. Materials and methods

2.1. Animals

Adult female Sprague–Dawley rats (250–275 g) from Charles River (Montreal, Canada) were habituated to the UBC colony for one week. The rats were pair-housed and maintained on a 12-h light–dark cycle (lights on at 07:00 h) under ambient temperature (22 ± 1 °C), with food and water available ad libitum. Approval by the UBC Animal Care and Use Committee was established for all procedures; the animals were treated in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

The dose of olanzapine (10 mg/kg) [Toronto Research Chemicals Inc., Toronto, ON, Canada] was carefully chosen to represent the middle-to-upper range of physiologically relevant levels in vivo, and is based on numerous previously reported studies that have examined the metabolic effects of olanzapine (Albaugh et al., 2006; Assie et al., 2008; Boyda et al., 2010b, 2012a, 2012b, 2013a). The vehicle solution for olanzapine was 50% polyethylene glycol 400, 40% distilled water and 10% ethanol (PEG solution). Olanzapine was administered by i.p. injection in a volume of 1 ml/kg, at 60 min prior to the glucose challenge. The doses of metformin (500 mg/kg, p.o.), rosiglitazone (30 mg/kg, p.o.) [Toronto Research Chemicals Inc., Toronto, ON, Canada] and glyburide (10 mg/kg, p.o.) [Sigma-Aldrich Inc., St. Louis, MO, USA] were based on prior preclinical studies (Hauton, 2011; Kiss et al., 2011) and our previous study that demonstrated these doses were sufficient to decrease olanzapine-induced hyperglycemia (Boyda et al., 2012a). The vehicle solutions for metformin and rosiglitazone were 0.9% saline, while the vehicle for glyburide consisted of PEG solution. Antidiabetic drugs were administered per os (gastric gavage) as a single daily administration for two consecutive days. The duration of antidiabetic drug treatment was set to two consecutive days to ensure that baseline fasting metabolic parameters (measured both before and after olanzapine administration) and postprandial measures could be studied following antidiabetic drug treatment. Solutions were compounded fresh daily, and all other chemical compounds were of reagent grade.

2.3. Baseline intraperitoneal glucose tolerance test (IGTT)

All rats were subjected to a baseline glucose tolerance test. Briefly, the animals were wrapped in a towel to minimize stress and a small drop of saphenous venous blood was procured with a 25 gauge needle for baseline blood glucose measurement at t = 0 min. All animals subsequently received a glucose challenge (1 g/kg/ml, i.p.) with repeated sampling of blood glucose readings at t = 15, 45, 75 and 105 min later. Blood glucose measurements were determined by a handheld glucometer (One Touch Ultra) as previously described (Boyda et al., 2010b, 2013b).

2.4. Antidiabetic drug treatment

Rats (n = 7–9 per group) were rank ordered based on performance in the baseline IGTT and then randomized into one of eight treatment groups: vehicle only, olanzapine and vehicle, olanzapine and metformin, olanzapine and rosiglitazone, olanzapine and glyburide, olanzapine and metformin + rosiglitazone, olanzapine and metformin + glyburide or olanzapine and rosiglitazone + glyburide. In the protocol, each rat received a single gavage administration of oral antidiabetic drug or vehicle on Day 1 at 11:00 h (see Fig. 1 — sequence of events). On Day 2, overnight fasted rats (16 ± 2 h) had their baseline blood glucose levels measured and then received a single intraperitoneal injection of olanzapine or PEG vehicle (t = 0 min). Sixty min later, the animals had a 100 μl saphenous blood draw; plasma was centrifuged (10,000 RPM, 10 min, 4 °C) and stored at −80 °C for analysis of insulin levels. The animals then received the second dose of antidiabetic drug or vehicle by gavage (i.e. 60 min post-olanzapine administration), followed by an i.p. challenge injection of glucose (1 g/ml/kg). Glucose levels were then measured every 15 min for the next 120 min. Each animal handler was blinded to drug treatment.

2.5. Insulin measurement by ELISA

Plasma samples extracted during Day 2 were analyzed for insulin levels using ultra-sensitive rat insulin Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (Crystal Chem Inc., IL, USA), and detection parameters as previously described (Barr et al., 2004, 2008; Boyda et al., 2010b). Briefly, 5 μl plasma samples were added and analyzed, in duplicate, on each 96 well plate according to the specific time points studied (t = 60 and t = 90 min). The samples were incubated at 4 °C for two hours followed by repeated washes. The substrate was added for 40 min and absorbance was measured at 450 nm–630 nm. Calibrators provided with the kit were used to generate a curve to interpolate insulin concentrations.

2.6. Statistical analysis

Variables were analyzed with a one-factor Analysis of Variance (ANOVA), with drug treatment as the between subjects factor, with alpha value set at $p < 0.05$. Individual glucose measurements during the eight time points during the IGTT were integrated to generate a single area under the curve value. The variables analyzed included: fasting levels of glucose prior to and at 60 min after the olanzapine drug challenge, the area under the curve (AUC) for the IGTT, and fasting