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Research report

Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats

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ABSTRACT

Second generation antipsychotics are commonly prescribed for the treatment of schizophrenia, however some can induce metabolic dysfunction side-effects such as weight gain, obesity and diabetes. Clinical reports suggest olanzapine alters satiety signals, although findings appear conflicting. Previous animal model studies have utilised a range of olanzapine dosages, however the dosage that better mimics the human scenario of olanzapine-induced weight gain is unclear. Female Sprague–Dawley rats were treated orally, three times daily with olanzapine (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), self-administered in a sweet cookie dough pellet at eight-hourly intervals) or vehicle (n = 12/group) for 14-days. Olanzapine orally self-administered in multiple doses (eight-hourly intervals) may circumvent a drop in plasma drug concentration and ensure the maintenance of a consistently high olanzapine level in the rat. Olanzapine increased body weight (0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), food intake (2.0 mg/kg) and feeding efficiency (0.5–2.0 mg/kg), with no effect on water intake. Subcutaneous inguinal (1.0 mg/kg, 2.0 mg/kg) and intra-abdominal perirenal fat were increased (2.0 mg/kg), but not interscapula brown adipose tissue. Olanzapine increased circulating ghrelin and cholecystokinin, but had no effect on peptide $YY_{(3-36)}$. Olanzapine decreased insulin (0.25–2.0 mg/kg) and locomotor activity in the open field arena (0.5–2.0 mg/kg). A low dosage of 0.25 mg/kg olanzapine had no effect on most parameters measured. Olanzapine-induced weight gain is associated with hyperphagia, enhanced feeding efficiency and adiposity, decreased locomotor activity and altered satiety signaling. The animal model used in the present study of self-administered oral olanzapine treatment (t.i.d.) at a dosage range of 0.5–2.0 mg/kg (but not 0.25 mg/kg) mimics aspects of the clinic.

1. Introduction

Compared to first generation classical antipsychotics, second generation antipsychotic drugs (SGAs) possess enhanced tolerability, with reduced risk of extra-pyramidal side effects, and an efficacy to treat multiple domains of schizophrenia [1–3], but are associated with numerous adverse side-effects including metabolic syndrome. Metabolic side-effects such as weight gain, adiposity, dyslipidaemia, glucose dysregulation and insulin resistance are particularly problematic, leading to further social and medical consequences including obesity, cardiovascular disease, type II diabetes and non-compliance to medication. In addition, the sedentary lifestyle predominant in schizophrenia patients [4–6] may further exacerbate the on-set of metabolic syndrome. Some potential mechanisms of SGA-induced weight gain have emerged in the past few years, including from our laboratory [7–11,12] and others ([13,14]; see reviews by [15,16]), however metabolism is a complex issue and an indisputable hypothesis remains elusive.

Olanzapine is particularly notorious for its weight gain side-effect [2,5], with reports indicating increases in body weight of 4.15 kg within 10-weeks of treatment [17] and 15.5 kg after 1-year [18] in some patients. Weight gain is associated with increased subcutaneous and intra-abdominal fat mass [19], with no effect on lean body mass [20,21]. Evidence suggests that olanzapine may alter appetite/adiposity hormones such as cholecystokinin (CCK), peptide YY (PYY), insulin and ghrelin. For example, chronic olanzapine treatment increases plasma insulin and induces insulin resistance in humans [22,23] and rats [24], even after a single acute dose of olanzapine, risperidone or clozapine [25,26]. Olanzapine may also alter CCK levels, as Vidarsdottir et al. [27] reported a slight preprandial morning elevation in plasma CCK levels in males, but no change in plasma PYY levels under these conditions. However, whether these results can be replicated in a rodent model is unknown. Reports on the effects of olanzapine on ghrelin levels in humans

Abbreviations: CCK, cholecystokinin; PYY, peptide YY; SD rat, Sprague–Dawley rat.

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are inconsistent [13,28–30] (also see [31] for review), which may be attributed experimental variables such as gender, body mass index at the time of testing, olanzapine dosage, length of treatment, concomitant treatments, history of illness and treatment [32], and a biphasic response of ghrelin to antipsychotic treatment duration [33]. In order to further study the effects of olanzapine on these important metabolic hormones there is a need for an appropriate animal model that closely resembles the human scenario of olanzapine-induced weight gain, including adiposity, locomotor activity and appetite signal interference, in the absence of the variables associated with some human studies.

For the past decade scientists have worked to establish an animal model that mimics human antipsychotic-induced weight gain, however inconsistencies between basic research and the clinic are a long-standing debate. Cocurrello and Moles [15] provide a recent review of rat and mouse models of SGA-induced metabolic dysfunction, and discussion on findings in the clinic compared to basic science. One of the issues overshadowing animal modeling of SGA-induced metabolic dysfunction is gender sensitivity, with reports of unsuccessful modeling of SGA-induced weight gain in the male rat [34,35,24,36,37]. However, some aspects of metabolic side-effect can be modeled in the male rat as risperidone induces body weight gain and hyperphagia in the male rat [38], acute olanzapine treatment increases food intake in a run-way paradigm [39], acute clozapine and olanzapine treatment, but not haloperidol, increases fat intake [40], and van der Zwaal et al. [101] recently reported increased food intake and meal size, and decreased locomotor activity in male rats after olanzapine treatment. Minet-Ringuet et al. [35] reported enhanced adiposity in male rats following olanzapine treatment, but no weight gain. However shortly after, the same group reported successful weight gain and increased adiposity in the male rat by altering some experimental conditions, i.e. 0.5 mg/kg, 2.0 mg/kg olanzapine dosage self-administered mixed with food [41]. These methods [41] were recently modified by Shobo et al. [42], who also reported success in modeling hyperphagia and enhanced adiposity in the male rat following chronic 5 mg/kg olanzapine mixed with food, and hyperphagia after an acute injection of 2 mg/kg olanzapine. The self-administration method of antipsychotic dosage may better mimic the oral route of drug administration in the clinic [43], and reduce stress response inevitable during daily subcutaneous or intraperitoneal injection and oral gavage [44].

Previous studies have reported olanzapine's ability to increase food intake, weight gain and adiposity in female rats at dosages ranging from 0.5 to 8 mg/kg [24,34,37,45–47], but not at 20 mg/kg/day [24,37]. Thus, the most appropriate dosage that better mimics the multiple aspects of human olanzapine-induced weight gain is unclear. Therefore, we examined the dose-dependent effects of olanzapine on food and water intake, body weight gain, abdominal and subcutaneous adiposity and locomotor activity, as well as circulating metabolic hormones, CCK, $PYY_{(3–36)}$, insulin and ghrelin in order to assist in understanding the dosage that best mimics aspects of the clinic. Additionally, the present study employed an oral self-administration method for olanzapine delivery, which resembles human drug administration, with a dosage of three times per day to ensure a consistently high concentration of olanzapine in the rat.

2. Experimental procedures

2.1. Animals and diet

Female SD rats (7 weeks old) were obtained from the Animal Resources Centre (Perth, WA, Australia), housed at 22 °C, on a 12-h light–dark cycle (lights on: 07:00 h), and allowed *ad libitum* access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) throughout the study. Animals were randomly assigned to one of the following treatments: 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg (Eli Lilly, Indianapolis, IN, USA), or vehicle ($n = 12$), three times daily at eight-hourly intervals. In the rat, the half-life of olanzapine is 2.5 h in the plasma and 5.1 h in the brain, however levels are still high after 8 h [48]. Therefore, in the present study rats were administered olanzapine three times/day to ensure a consistently high drug concentration to better mirror the human scenario of oral administration once per day. Following 1 week habituation, animals underwent a teaching period to self-administer a sweet cookie dough pellet three-times per day for 1 week, and were sham-weighed daily to minimise handling stress during treatment.

Cookie dough (62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) administration methods were employed as previously reported by our laboratory [10]. Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%) was produced. Olanzapine tablets were separated from their coating, then pulverized using a mortar and pestle. The assigned dosage of powdered olanzapine was added to the measured dry ingredients. Water was added to achieve a dry-dough consistency immediately prior to administration and a 0.3 g cookie-dough pellet (containing the assigned olanzapine dosage) was offered by metal spoon at eight-hourly intervals (three pellets/day) for 14-days. Animals were observed during treatment administration to ensure complete consumption of each pellet, and were weighed daily approximately mid-way through their wake cycle. Food and water intake were recorded daily and results were corrected for spillage. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Behavioural analysis: open field testing

On treatment days 8–10, animals were subjected to open field behavioural testing to examine locomotor activity, as previously described by our group [49]. Briefly, a single animal was placed in the centre of a black square arena (60 cm × 60 cm wide, 40 cm high walls, 100 lx). Animal behaviour was recorded for 30-min and analysed using Ethovision video-tracking software (Nodulus Information Technology, Wageningen, The Netherlands). Central and peripheral duration (s) and frequency, as well as total distance travelled (cm), mean velocity (cm/s) and rearing data were recorded.

2.3. Hormonal and post mortem adiposity measurements

Animals were fasted for 4–6 h, then euthanized with sodium pentabarbitone. Upon sedation, blood was removed from the left ventricle for hormonal testing (CCK, $PYY_{(3–36)}$, insulin and total ghrelin). Samples for each hormone were separately collected in tubes containing 2Na-EDTA, aprotinin, K_3EDTA or heparin, immediately centrifuged, and plasma was aliquoted then stored at −20 °C. Fasting circulating insulin levels were measured using commercially available ELISA kits (Linco Research, MO, USA), whilst RIA kits were used to detect CCK, $PYY_{(3–36)}$ (Phoenix Pharmaceuticals, CA, USA) and total ghrelin (Linco Research, MI, USA) levels.

Inguinal, perirenal and periovary white fat pads, as well as sub-scapula brown fat pads, were dissected and individually weighed.

2.4. Data analysis

Data were statistically analysed using SPSS (version 15, SPSS, Chicago, IL, USA). One-way ANOVAs were used to observe the dosage response of olanzapine on total body weight, food and water intake, adipose tissue, insulin, CCK and $PYY_{(3–36)}$. Two-way repeated ANOVAs (DOSAGE × DAYS as repeated measures) were employed for cumulative weight gain, food and water intake. Multiple comparisons were performed using post hoc Dunnett-*T* tests. Where Kolmogorov–Smirnov tests revealed abnormal data distribution, Kruskall–Wallis tests were utilised followed by Mann–Whitney *U* post hoc analysis. Correlations were identified using Pearson's correlation tests or Spearman's correlation tests for non-parametric data. Data was considered significant when $p < 0.05$.

3. Results

3.1. Body weight

A one-way ANOVA revealed a significant effect of olanzapine dosage on total body weight gain ($F_{4,55} = 7.25$, $p < 0.01$). Post hoc analysis identified a significant increase in total body weight following 0.5 mg/kg and 1.0 mg/kg ($p < 0.05$) olanzapine/kg compared to controls, with the highest increase (+45%) observed in the 2.0 mg/kg treatment group ($p < 0.01$), but no change in the 0.25 mg/kg group (Table 1). A two-way repeated ANOVA (DOSAGE × DAYS) of cumulative body weight gain revealed significant effects of dosage ($F_{4,55} = 6.40$, $p < 0.01$) and days ($F_{13,715} = 331.78$, $p < 0.01$), and a significant interaction between the two factors ($F_{52,715} = 7.61$, $p < 0.01$). Cumulative weight gain was