A Comparison of the Effectiveness of Metformin and Acetaminophen in Preventing Olanzapine Toxicity in Mice

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Abstract: Background: Olanzapine (OLZ), an atypical antipsychotic drug, causes weight gain and diabetogenic effects in a subset of patients. Conventional anti-diabetic drugs such as Metformin (MET) are often prescribed to reduce these effects. Recent evidence suggests that acetaminophen (APAP) may be an effective alternative to MET in patients receiving OLZ. The purpose of this study was to directly compare APAP, MET and APAP plus MET in the prevention of metabolic changes due to OLZ in C57BL/6J mice consuming a High Fat (HF) diet. Materials and Methods: Body weight gain, body fat percentage, glucose tolerance, plasma insulin levels, insulin resistance and glycosylated hemoglobin levels were measured and analyzed. Mice received a HF diet and tap water supplemented with OLZ (3 mg kg BW⁻¹ d⁻¹). Mice also received APAP (25 mg kg BW⁻¹ d⁻¹), MET (200 mg kg BW⁻¹ d⁻¹) or APAP+MET. Results: APAP significantly reduced fasting glucose levels by 30%. In OLZ-treated mice, MET reduced the total body fat percentage (17%), impaired glucose tolerance (64%), fasting blood glucose (14%), insulin resistance (26%) and glycosylated hemoglobin levels (42%). Combined treatment with APAP+MET was more effective than either treatment alone in reducing rate of body weight gain (29%), fasting blood glucose (40%) and insulin resistance (36%). However, APAP elevated chronic blood glucose levels by 25%, estimated as glycosylated hemoglobin. Conclusion: Overall, MET appeared to be the better drug in the prevention of OLZ-induced pre-diabetic changes than APAP. While treatment with APAP+MET may improve some pre-diabetic risk factors, concerns regarding the effect APAP on chronic levels of blood glucose may limit its clinical relevance.

Key words: Acetaminophen, high fat diet, insulin resistance, metformin, mice, olanzapine

INTRODUCTION

Obesity results from an imbalance in energy intake and expenditure, typically due to a sedentary lifestyle and High Fat (HF) and high caloric diets. Certain atypical antipsychotic drugs, such as olanzapine (OLZ; 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzo[d]azepine) are also associated with increased risk for weight gain (Nasrallah, 2003; Newcomer et al., 2002). OLZ was approved by the U.S.FDA in 1996 to treat schizophrenia, bipolar disorder and other psychotic conditions. Associated with the OLZ-mediated gain in body weight and fat are increases in insulin resistance, hyperglycemia and hyperlipidemia which are risk factors for the development of Type II Diabetes Mellitus (T2DM) and cardiovascular disease (Bray, 2004; Newcomer et al., 2002). Such adverse metabolic effects produce a high rate of attrition in patients receiving OLZ (Lieberman et al., 2005).

Metformin (MET; N, N-dimethylimidodicarbonimidic diamide) is an oral biguanide drug widely used as an antihyperglycemic in the treatment of T2DM or in conditions that might lead to hyperglycemia (e.g., the use OLZ) (Chen et al., 2008; Wu et al., 2008). Since MET lowers blood glucose without increasing insulin production, it is considered to be an insulin sensitizer (Stumvoll et al., 1995). The peripheral effect of MET would explain the lack of stimulation of insulin secretion in its antihyperglycemic action. Since the hyperinsulinemia and insulin resistance induced by some other antihyperglycemic drugs are not seen with MET, MET is considered to be a safe and effective drug for the treatment of hyperglycemia and T2DM.

Acetaminophen (APAP; N-(4-hydroxyphenyl) acetamide) is an over-the counter analgesic and antipyretic that has antioxidant properties (Merrill, 2002; Orhan and Sahin, 2001). APAP is inexpensive and side effects are rare when used at recommended dosages.
mice on a HF diet or a HF diet supplemented with OLZ, APAP decreased body fat percentage and normalized glucose and insulin homeostasis (Kendig et al., 2008; Shertzer et al., 2008, 2010).

The purpose of this study was to directly compare APAP to MET in ability to prevent adverse metabolic changes due to the administration of OLZ in mice consuming a its HF diet. We hypothesized that APAP would either have efficacy comparable to MET or improve the efficacy of MET, in minimizing adverse metabolic changes associated with OLZ treatment. If this hypothesis proved to be correct, APAP could offer an inexpensive alternative or adjuvant therapy with conventional anti-diabetic drugs used to treat metabolic disorders.

**MATERIALS AND METHODS**

**Chemicals:** OLZ was purchased from Molcan Corporation, (Ontario, CA) with 98.9% purity. Metformin hydrochloride (MET) was purchased from Torris Bioscience (Ellisville, MO, USA). All other chemicals and reagents were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

**Animals and treatment:** All animal experiments were conducted in accordance with the National Institutes of Health standards for care and use of experimental animals and the University of Cincinnati Animal Care and Use Committee. Female C57BL/6j mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA), weighed and assigned to treatment groups. Mice were maintained on a 12 h light-dark cycle and allowed ad libitum access to tap water containing OLZ and a HF diet containing 40% energy derived from fat, with 7.74 kJ fat energy per gram of diet (Research Diets, Inc., New Brunswick, NJ, USA). OLZ was prepared weekly and administered at (3 mg kg BW⁻¹ d⁻¹). The concentration of OLZ in the drinking water was approximately 25 μg mL⁻¹, utilizing the average water consumption of mice for the previous week (approximately 120 mL kg BW⁻¹ d⁻¹). In addition to OLZ, tap water for the intervention groups contained APAP (0.25 mg APAP mL⁻¹) or MET (2 mg MET mL⁻¹), such that the calculated daily dosages per kg of BW were approximately 25 mg APAP and 200 mg MET. The duration of the treatments was 10 weeks, with weekly measurements of Body Weight (BW) and food and water consumption.

**Procedures with live, unanesthetized mice:** Glucose concentration was determined with a handheld glucometer (Ascensia Contour glucometer, Bayer HealthCare LLC, Mishawaka, IN, USA). Glucose tolerance tests were performed after an 8 h fast. Samples of blood (5 μL) from the great saphenous vein were applied directly to the glucose strip to measure Fasting Blood Glucose levels (FBG). After initial FBG measurements, 1.5 mg D-glucose/g body weight was administered by intraperitoneal injection, followed by blood glucose determinations at 20 min intervals for 120 min. Plasma insulin was determined with the Insulin (Mouse) Ultrasensitive Enzyme Immunoassay (ALPCO Diagnostics, Salem, NH, USA) according to manufacturer’s instructions. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR), calculated as the product of plasma insulin levels (μU mL⁻¹) X blood glucose concentration (mg dL⁻¹) in feed-deprived mice (Andricopoulos et al., 2008). Body composition was assessed by nuclear magnetic resonance (EchoMRI, EchoMedical Systems, Houston, TX, USA), to provide estimates of total fat tissue, lean tissue (muscle) and water (Tinsley et al., 2004).

**Glycosylated hemoglobin:** The percentage of hemoglobin that is glycosylated (HbA1c), a measure of average chronic blood glucose levels, was determined with a Diazyme Direct Enzymatic HbA1c Assay kit (Diazyme Laboratories, Poway, CA, USA) according to the manufacturer’s instructions.

**Statistical analysis:** Statistical significance of the differences between group sample mean values was determined by 2-way or 3-way ANOVA, followed by the Student-Newman-Keuls test for pairwise comparison of means. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

In this study, MET and APAP, alone or in combination, generally improved physiological parameters associated with the risk for developing T2DM in mice receiving a HF diet and treated with the antipsychotic drug OLZ. Linear regression analyses were performed on weekly body weight gains for each mouse and the mean body weight gains per day were used to evaluate statistical differences between groups. While there was no significant different in the amount of food intake between treatment groups (negative data not shown), there was a significant 29% decrease in the rate of body weight gain of mice in the MET+APAP group as compared to OLZ with no treatment group (Fig. 1). The effect of MET in lowering the rate of gain in body weight is consistent with human trials in schizophrenic patients receiving OLZ and MET (Chen et al., 2008; Klein et al., 2006; Wu et al., 2008).
Fig. 1: Cumulative percent increases in BW. The 100% value for BW gain for OLZ mice was 0.66±0.05%/d. Values are Mean %±SEM relative to OLZ, n = 4. *Different from OLZ, p<0.05.
†Different from OLZ+MET, p<0.05

The amount of lean, calculated either as [lean weight/(BW minus body fat)] or as [(b.wt. minus body fat)/body water], was not changed for any treatment group (negative data not shown). The average body fat of OLZ mice receiving no treatment was 31% at the end of the study. Slight decreases in percentage of body fat compared to the OLZ group with no treatment were found for mice supplemented with APAP (10%), MET (17%) or APAP+MET (19%) (Fig. 2). Since the amount of lean for each group was the same, the differences in weight gain among treatment groups can be attributable to changes in overall fat mass. The lack of effect of APAP on body weight gain and the small reduction in body fat accumulation in mice treated with OLZ contrasts with a previous report from this laboratory (Shertzer et al., 2010) which showed that APAP afforded a reduction in OLZ-mediated gains in body weight and fat. Two differences in the two studies could account for the differing results. First, mice in the current study averaged 15% lower initial body weights than the previous study. Second, mice in the present study drank about one-third less water, such that the dosage for APAP was lower. Since total body fat is composed of both visceral and subcutaneous adipose tissue, a small difference in mouse size or APAP dosage may differentially affect the two fat compartments. Visceral adiposity is more strongly associated with increased metabolic disease including cardiovascular disease and T2DM than total adiposity (Hamdy et al., 2006). Additional studies are needed to determine the effects of APAP and MET on the distribution of visceral and subcutaneous fat.

In addition to increased body fat, metabolic risk factors for the development of T2DM include elevated FBG, impaired glucose tolerance and insulin resistance. In this study, elevated FBG levels are the primary indicator of T2DM. Compared to mice receiving only OLZ, significant reductions were observed in FBG levels in mice also treated with APAP (30%), MET (14%) and APAP+MET (40%) (Fig. 3). The net reduction in FBG by APAP+MET appeared to be the sum of reductions by APAP and MET, with APAP having the greater contribution. Because impaired glucose tolerance is a pre-disposing factor for T2DM, we performed a Glucose Tolerance Test (GTT) at the conclusion of the treatment period. The Area Under the Curve (AUC) for blood glucose against time showed that, compared to OLZ with no additional treatment, MET and APAP+MET significantly improved glucose tolerance by 64 and 30%, respectively (Fig. 4). Both animal (Shertzer et al., 2010) and clinical trials (Baptista, 1999; Newcomer et al., 2002) have shown an association between OLZ treatment and impaired glucose tolerance. Newcomer et al. (2002) evaluated glucose tolerance in schizophrenic patients receiving atypical antipsychotics, including OLZ and in untreated healthy control subjects. OLZ treated
Fig. 3: Fasting blood glucose. Fasting blood glucose (FBG) was determined after an 8 h fast. The 100% value for OLZ was 154±4 mg dL⁻¹. Values are Mean %±SEM relative to OLZ, n = 4. *Different from OLZ, p<0.05. †Different from OLZ+MET, p<0.05.”

Fig. 4: Glucose homeostasis. Glucose tolerance, expressed as the area-under-the-curve (AUC) for blood glucose versus time for 120 min after a glucose challenge. The 100% value for OLZ was 25, 180±6.381 min (mg dL⁻¹). Values are Mean %±SEM relative to OLZ, n = 4. *Different from OLZ, p<0.10. †Different from OLZ+MET, p<0.05.”

Fig. 5: Insulin homeostasis. Insulin resistance was calculated as HOMA-IR. The 100% value for OLZ was 5.39±0.04. Values are Mean %±SEM relative to OLZ, n = 4. *Different from OLZ, p<0.05. †Different from OLZ+MET, p<0.05.”

patients had significantly higher blood glucose levels, compared to untreated controls. In clinical studies, MET treatment was able to reverse the HF diet+OLZ-induced glucose intolerance (Chen et al., 2008; Klein et al., 2006), similar to findings in the present study. Unfortunately, in this study, APAP was neither able to improve glucose tolerance by itself, nor improve the efficacy of MET for glucose tolerance, in HF+OLZ treated mice.

An important clinical diagnostic indicator of the risk for developing T2DM is an increase in fasting plasma insulin levels. In mice as well as humans, a HF diet is associated with an increase in fasting plasma insulin levels (Bray, 2004; Shertz et al., 2008, 2009) and OLZ exacerbated the increase in fasting plasma insulin (Shertz et al., 2010). Fasting glucose and insulin levels can be used to estimate insulin resistance, using the HOMA-IR model which we used in this study. APAP did not reduce HOMA-IR from values obtained in mice treated with OLZ alone. However, significant decreases in insulin resistance were obtained with MET (26%) and APAP+MET (34%) (Fig. 5). We also used glycosylated hemoglobin (HbA1c) to estimate chronic blood glucose levels. While FBG and glucose tolerance represent a specific time point, glycosylated hemoglobin (HbA1c) represents a measure of average blood glucose levels during the half-life of the RBC which for mice is about 20 days (DeLouch and Droleskey, 1986). Although HbA1c
Fig. 6: Chronic glucose homeostasis. Glycosylated hemoglobin is expressed as HbA1c. The 100% value for OLZ was 4.26±0.21%. Values are Mean ±SEM relative to OLZ, n = 4. a Different from OLZ, p<0.05. b Different from OLZ+MET, p<0.05.

levels were not unusually high in the HF+OLZ treatment group, APAP significantly increased HbA1c by 25%, whereas MET and APAP+MET significantly decreased HbA1c levels by 42 and 16%, respectively (Fig. 6). This surprising finding that APAP could actually increase the value for HbA1c could limit the clinical applicability of APAP for use in normalizing glucose homeostasis.

A high fat diet is known to cause increases in insulin resistance, followed by a somewhat delayed impairment of insulin secretion by pancreatic β-cells (Bray, 2004). The resulting elevated blood glucose levels can produce tissue damage, due in part to hyperglycemia-induced oxidative stress (Vincent et al., 2005). Present results showed that APAP significantly reduced the insulin resistance associated with HF+OLZ treatment. We previously proposed that in mice consuming a HF diet, APAP protects from insulin resistance and loss of β-cell function via its activity as an antioxidant and reactive oxygen scavenger (Sherzer et al., 2008). Other studies support the ability of APAP to protect against oxidative stress and resulting tissue damage. Merrill (2002) showed that APAP is capable of inhibiting H$_2$O$_2$-induced lipid peroxidation in erythrocyte membranes in vitro (Merrill, 2002). Orhan and Sahin (2001) demonstrated that ischemia-mediated protein oxidation by peroxynitrite and H$_2$O$_2$ was reduced by APAP in guinea pig myocardium (Orhan and Sahin, 2001). It is, therefore, likely that the protection afforded by APAP in HF+OLZ-treated mice results, at least in part, by reducing oxidative stress.

In conclusion, elevated amounts of dietary energy and fat contribute to the increasing incidence of obesity and related health issues, including T2DM and cardiovascular diseases. The widely prescribed antipsychotic drug OLZ may exacerbate the development of risk factors leading to metabolic diseases, resulting in the use of secondary medication, such as the highly efficacious drug MET, to regulate blood glucose. These animal studies suggest that APAP may augment the ability of MET to improve certain metabolic parameters associated with the risk for developing T2DM. However, the potential for APAP to increase chronic blood glucose levels limits its potential for utilization in mitigating metabolic diseases associated with obesity or a HF diet.

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