Metformin: An effective attenuator of risperidone-induced insulin resistance hyperglycemia and dyslipidemia in rats

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The use of atypical antipsychotics in the clinical management of schizophrenia and schizoaffective disorders has been associated with the development of insulin resistance. The present study evaluates the possible individual ameliorating effect of single daily oral treatments with 20 mg/kg/day of metformin and 0.1 mg/kg of glibenclamide in two groups of Wistar rats pretreated with 0.2 mg/kg of risperidone for 60 days. Two additional groups of rats were only treated with 0.2 mg/kg of risperidone and 10 mL/kg of distilled water, respectively, also for 60 days. Results showed that oral pre-treatment with metformin significantly attenuated increases in the weight gain pattern, fasting glucose, fasting plasma insulin, serum triglyceride and total cholesterol levels that were elevated by risperidone treatment. Metformin also significantly reduced glycosylated hemoglobin concentration, fasting insulin-glucose ratio and fasting insulin resistance index. Conversely, oral pre-treatment with glibenclamide for 60 days did not significantly reduce any of the measured parameters except for glycosylated hemoglobin concentrations. Thus, results of this study showed that 20 mg/kg of metformin effectively ameliorated the development of risperidone-induced insulin resistance and dyslipidemia which was mediated via improvement in insulin resistance. This study provides insight into the therapeutic potential of metformin in preventing risperidone-induced insulin resistance diabetes mellitus and dyslipidaemia.

Keywords: Glibenclamide, Insulin resistance, Metformin, Rats, Risperidone

The increasing global prevalence of schizophrenia among the adult population has resulted in an increasing use of antipsychotics, particularly, the atypical antipsychotics, in the effective clinical management of schizophrenia. Atypical antipsychotics are also employed in the management of other psychiatric conditions such as bipolar disorders and Alzheimer’s disease. However, schizophrenia is associated with high morbidity and high mortality resulting from strikingly high suicide rate of 10%1,2,3, obesity, dyslipidemia4 and accelerated heart disease (which is two to three times higher than that of the general population)1,5,6.

The introduction of chlorpromazine in the late 1950s transformed and formed a new frontier in the clinical management of schizophrenia. During this era several major chemical classes of antipsychotic drugs, later termed “conventional neuroleptics”, were developed. These classes include the phenothiazines (e.g. chlorpromazine), the butyrophenones (e.g. haloperidol), and the thioxanthenes (e.g. flupenthixol). Although, these agents effectively controlled and improved the schizophrenic symptomology but were also associated with major drawbacks, which on their own contributed stigma to the already existing schizophrenia7,8. Their side-effects include Parkinsonian-like movement-disorders (“extra pyramidal side effects” resulting from antagonism at dopamine D2 receptors in the basal ganglia)9, excessive daytime somnolence, dry mouth, blurred vision, constipation, glaucoma (particularly in the elderly patients)9, endocrine disorders (such as hyperprolactinemia, sexual dysfunction, and infertility) and metabolic disorders (obesity, dyslipidemia and insulin resistance)4. As a
result of these side-effects, newer neuroleptics (termed “atypical antipsychotics”, e.g. clozapine, olanzapine, risperidone, quetiapine, Ziprasidone, etc.) with lower risk of extrapyramidal side-effects were introduced into clinical use for the management of schizophrenia. However, these newer antipsychotics was fast reported to be associated with development of glucose intolerance and/or overt type 2 diabetes mellitus. Although it remains very controversial if schizophrenia is strongly linked with type 2 diabetes or it is the use of atypical antipsychotics that results in over type 2 diabetes.

For several decades, metformin (a biguanide), and the second generation sulfonylurea, glibenclamide, have been widely used and are still employed in the clinical management of type 2 diabetes untreatable with diets alone. Metformin controls hyperglycemia by enhancing peripheral glucose utilization particularly in the hepatic and skeletal muscles while the sulfonylureas induce hypoglycaemia by stimulating insulin release from the pancreatic β-cells. In view of the increasing incidence of antipsychotic-induced insulin resistance hyperglycemia and dyslipidemia, particularly with prolonged risperidone use and lack of effective therapeutic measures at stemming down these metabolic derangements, the present study has been designed at evaluating the possible therapeutic potential and mechanism(s) of action of clinical doses of metformin (20 mg/kg/day) and glibenclamide (0.1 mg/kg/day) in ameliorating the development of insulin resistance in rats repeatedly treated with single daily oral dose of 0.2 mg/kg of risperidone for 60 days.

Materials and Methods

Drugs and chemicals—Risperidone (Ris-2®, Ind-Swirf Ltd. (Unit-1), Parwanoo, India), glibenclamide (Daonil®, Hoechst Marion Roussel Limited, Mumbai, India) and metformin (Glucophage®, Merck Marker (Pvt.) Ltd., Quetta, Pakistan) were used.

Experimental animals and their care—Young adult male Wistar rats (20; age: 10-12 weeks, weight: 110-130 g) were obtained from the rat colony of the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria, after an ethical approval has been obtained. The rats were handled in accordance with international principles guiding the use and handling of experimental animals. The rats were maintained on standard rat feed (Ladokun Feeds, Ibadan, Nigeria) and tap water which were made available ad libitum. The rats were maintained at an ambient temperature between 28°C-30°C, 55 ± 5% RH, and standard (natural) photoperiod of approximately 12 h of light (06:30–18:30 hrs) alternating with approximately 12 h of darkness (18:30–06:30 hrs). The study was conducted at the Animal Experiment Facility in the Animal House of Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria, between January and March, 2010.

Treatment of animals—The rats were randomly divided into 4 groups of 5 rats each in such a way that the weight difference between and within group does not exceed ±20% of the average weight of the sample population. Group I rats served as the untreated control and were orally pre-treated with 10 mL/kg distilled water. Group II rats were pre-treated with 10 mL/kg of distilled water 1 h before 0.2 mg/kg of risperidone while Groups III and IV rats were pre-treated with 20 mg/kg of metformin and 0.1 mg/kg of glibenclamide 1 h before oral treatment with 0.2 mg/kg of risperidone, all dissolved in distilled water. All oral treatments lasted 60 days and all the rats were handled under the same sham-handling.

Body weight measurement—In the course of the 60-days oral treatment, body weights of rats were regularly taken at 15 days interval with electronic Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). Absolute and percentage (%) weight changes were calculated in respect of the initial body weight on day 1.

Blood collection and bioassays—After an overnight fast on day 60, rats were deeply anesthetized with inhaled halothane and paired samples of whole blood were drawn directly from the heart chamber. Blood samples were collected into heparinized and plain bottles. The heparinized samples were analyzed for free plasma insulin and glycosylated hemoglobin while blood samples collected into the plain bottles were analyzed for serum triglyceride and total cholesterol. The blood samples were kept at room temperature (23°C) for 6 h to allow for complete clotting before they were centrifuged at 3000 rpm for 20 min to obtain a clear serum. The individual separated serum was pipetted into correspondingly labelled vial for triglyceride and cholesterol assays.

Measurement of fasting blood glucose in the treated rats—The fasting blood glucose (FBG) concentrations in rats were first determined on day 1.
before oral drug treatment was initiated and on day 61 after termination of drug treatment. Blood sample from the rat tail vein for fasting whole blood glucose was collected by tail tipping method. The tail was gently squeezed to let out 2-3 drops of fresh whole blood which were placed on the test spot of the glucose strip after which the test strip was gently inserted into Test Strip Platform of the Microprocessor digital blood glucometer and the readings were recorded. The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment, using glucose oxidase method on a One Touch Basic Blood Glucose Monitoring System® (LifeScan Inc., Milpitas, California, U.S.A.) as previously adopted by Adeneye et al.

Measurement of plasma free insulin—Plasma insulin concentration was estimated in duplicate using double-antibody radioimmunoassay (RIA) kits, INSIK-5 (Diasorin, Italy), with 125I-labelled insulin as tracer on a gamma counter. The sensitivity of this assay is 2 pM, the intra- and inter assay coefficients of variation are 5 and 7%, respectively.

Measurement of plasma glycosylated hemoglobin—Glycosylated hemoglobin (HbA1c) of collected blood sample was determined using mobile affinity electrophoresis procedure as described by Ambler et al. Briefly, about 100 µl of heparinized whole blood was added to 500 ml of sodium chloride solution in a centrifuge tube, mixed gently, and centrifuged at 2500 rpm for 10 min. The supernatant fluid was aspirated as completely as possible and 350 µl of the hemolysing solution was added. Membranes of the electrophoresis machine (Seprateck Electrophoresis Chamber, Gelman Sciences Inc., Ann Arbor, MI 48106) were equilibrated with affinity electrophoresis buffer (“Glyco-Phore Buffer”, Gelman Sciences, production number 51261) for at least 10 minutes and chambers filled with buffer. The affinity electrophoresis buffer of concentration 33 mmol/l was constituted as 2 µmol of dextran sulphate and 8 µmol of disodium EDTA per litre. The applicator wells were loaded with hemolysed samples (7 µl per sample well) by the use of a Gilson “Pipetman”. The membranes were blotted and placed in the chamber and the sample applied. To ensure an even application, the applicator was not removed from the membrane until the sample was totally absorbed. After electrophoresis at 150 Volts for 40 min the strips were promptly placed in protein stain (5 g/l of Ponceau S in 75 g/l of trichloroacetic acid solution) for 10 min. Excess stain was washed out by constantly agitating in changes of 50 mL/l acetic acid for a maximum of 5 min. The membranes were cleared in “Super Sepraclear” clearing solution for 2 min, transferred to a glass plate, and placed in an oven at 80°C-90°C for about 10 min until transparent. The separations were scanned from the anode with an ACD-18 Computing Densitometer at a wavelength of 520 nm. The corresponding wavelength obtained for each sample was compared with that of the standard and the HbA1c estimate was extrapolated.

Measurement of serum triglyceride and total cholesterol—The serum triglyceride (TG) and total cholesterol (TC) were estimated by enzymatic methods using analytical kits (Biolabo SA, Maizy, France). All the estimations were carried out according to kit manufacturers’ instructions.

Determination of glycemic indices—The glycemic indices: fasting insulin glucose ratio (FIRG) and fasting insulin resistance index (FIRI) were calculated using methods of Harati et al. and Shalam et al., respectively. Mathematically, these formulae are expressed as:

\[ \text{FIRG} = \frac{\text{fasting insulin}}{\text{fasting glucose}} \]

\[ \text{FIRI} = \frac{(\text{fasting insulin} \times \text{fasting glucose})}{25} \]

Statistical Analysis—Results were presented as mean ± S.D. for body weights while data for biochemical parameters were expressed as mean ± S.E. of five observations. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-Newman-Keuls test on SYSTAT 10.6. Statistical significance were considered at \( P<0.05 \), \( P<0.01 \), and \( P<0.001 \).

Results

Effect of prolonged metformin and glibenclamide treatments on the pattern of weight gain in risperidone-treated rats—Prolonged treatment with 0.2 mg/kg risperidone alone resulted in significant time-dependent increases in the weight gain pattern, particularly from day 30 of treatment with risperidone (Fig. 1). With 20 mg/kg of metformin pre-treatment, there was a significant reduction in the weight gain pattern in the treated rats. A converse effect was observed with glibenclamide pre-treatment as glibenclamide treatment was observed to significantly increased the weight gain pattern of treated rats from the day 15 to 61 days of treatment (Fig. 1).
Effect of metformin and glibenclamide on the fasting blood glucose concentration in risperidone-treated rats—Repeated oral treatment with 0.2 mg/kg of risperidone for 60 days resulted in a significant rise in the fasting glucose level when compared to the basal values on day 1 and Group I values on day 61 (Fig. 2). This elevation was significantly attenuated by oral pre-treatment with 20 mg/kg of metformin. However, pre-treatment with glibenclamide did not significantly attenuate a rise in the fasting blood glucose (Fig. 2).

Effect of metformin and glibenclamide treatments on the fasting plasma insulin and glycosylated hemoglobin (HbA$_1c$), serum triglyceride and total cholesterol concentrations in risperidone-treated rats—Prolonged oral treatment with single daily oral dose of 0.2 mg/kg of risperidone resulted in significant increase in the circulating levels of fasting free plasma insulin and significant increase in the glycosylated hemoglobin concentration (Table 1). However, these increases were significantly attenuated by metformin pre-treatment. Although pre-treatment with 0.1 mg/kg glibenclamide significantly attenuated an increase in the plasma level of HbA$_1c$ but has no significant attenuating effect on the circulating free insulin concentration (Table 1). In addition, prolonged oral treatment with 0.2 mg/kg of risperidone resulted in non-significant elevations in the serum concentrations of triglyceride and total cholesterol. While these elevations were significantly attenuated with metformin pre-treatment, glibenclamide pre-treatment caused no significant elevation in the serum levels of triglycerides and total cholesterol (Table 1).

Effect of 60 days of metformin and glibenclamide treatments on glycemic indices in risperidone-treated rats—Single, daily oral treatment with 0.2 mg/kg of risperidone resulted in significant increases in the glycemic indices-FIGR and FIRI (Table 1). In the same vein, oral pre-treatment with 20 mg/kg of metformin significantly reduced the glycemic indices in the treated rats. However, pre-treatment with 0.1 mg/kg of glibenclamide for 60 days further significantly increased the FIGR values, an increase that is even significantly higher than those treated with risperidone alone (Table 1). Similarly, oral treatment with glibenclamide significantly increased the FIRI value when compared to untreated control values (Table 1).

Discussion

The atypical antipsychotic agents have become the preferred treatment for schizophrenia and schizoaffective disorders$^{27}$ as they are comparatively...
Table 1—Effect of 60-days of oral treatment of 20 mg/kg metformin and 0.1 mg/kg glibenclamide on the fasting plasma insulin (FPI), fasting insulin-glucose ratio (FIGR) and fasting insulin resistance index (FIRI), glycosylated hemoglobin (HbA1c), serum triglyceride (TG) and total cholesterol (TC) in risperidone-treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gr. I</th>
<th>Gr. II</th>
<th>Gr. III</th>
<th>Gr. IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPI (pM/L)</td>
<td>53.20±3.57</td>
<td>154.20±8.55</td>
<td>58.00±4.72</td>
<td>154.40±3.47</td>
</tr>
<tr>
<td>FIGR</td>
<td>0.78±0.02</td>
<td>1.27±0.05</td>
<td>0.75±0.04</td>
<td>1.40±0.07</td>
</tr>
<tr>
<td>FIRI</td>
<td>145.77±15.44</td>
<td>751.38±59.76</td>
<td>179.27±21.22</td>
<td>685.78±71.43</td>
</tr>
<tr>
<td>HbA1c</td>
<td>3.32 ± 0.36</td>
<td>6.28 ± 0.61</td>
<td>3.34 ± 0.35</td>
<td>4.68 ± 0.41</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.65 ± 0.05</td>
<td>2.01 ± 0.06</td>
<td>0.94 ± 0.11</td>
<td>1.95 ± 0.14</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>2.46 ± 0.12</td>
<td>3.08 ± 0.19</td>
<td>2.02 ± 0.07</td>
<td>2.76 ± 0.29</td>
</tr>
</tbody>
</table>

b and c represent significant increases at P<0.01 and <0.001, respectively, when compared to untreated control (Gr. I) values; a represents a significant increase at P<0.05 while d,e and f represent significant decreases at P<0.05, <0.01, and <0.0001 respectively, when compared to Gr. II values. Gr. I = 10 mL/kg of distilled water; Gr. II = 0.2 mg/kg of risperidone; Gr. III = 0.2 mg/kg of risperidone + 20 mg/kg of metformin; Gr. IV = 0.2 mg/kg of risperidone + 0.1 mg/kg of glibenclamide

The present study evaluates the possible prophylactic potential of clinical doses of two classes of oral hypoglycemic drugs (metformin—a biguanide and glibenclamide—a second generation sulfonylureas) in attenuating the onset of insulin resistance in rats on prolonged risperidone treatment. Glibenclamide lowers blood glucose by 3 major mechanisms including increasing pancreatic release of insulin, decreasing hepatic clearance of insulin and stimulating the synthesis and expression of glucose transporters in the hepatic and skeletal muscles. On the other hand, metformin lowers blood glucose levels primarily by decreasing hepatic gluconeogenesis and enhancing insulin action in skeletal and adipose tissues, as well as inhibiting intestinal glucose uptake. Metformin has been widely reported to promote weight loss and effectively reduced plasma glycosylated hemoglobin by 2% and triglyceride by 15% to 20%.

In the present study, prolonged risperidone treatment caused profound weight in the treated rats which is in consonance with previous reports. The profound weight reduction induced by metformin pre-treatment suggests that metformin could have a weight-gain-attenuating effect on risperidone therapy as metformin has been previously reported to cause weight loss and improve insulin resistance in type 2 diabetes. Similarly, metformin was observed to have prevented the onset of hyperglycemia, dyslipidemia and hyperinsulinemia in risperidone-treated rats as indicated by reducing the circulating fasting glucose, triglyceride and total cholesterol levels, plasma insulin levels and glycemic indices. Again, these findings are in consonance with the earlier reports made by Matthaei et al. and Abbasi et al. that metformin improves insulin resistance in type 2 diabetes models. Conversely, glibenclamide at clinical dose has no therapeutic importance in this respect. Thus, summing the anti-hyperlipidemic effect of metformin and its weight-gain-attenuating effect, the weight losing mechanism of metformin in risperidone-treated rats is probably mediated via inhibition of lipogenesis while its antihyperglycemic effect is mediated via improvements in insulin resistance.

In conclusion, results of the present study showed the therapeutic potential of metformin in ameliorating risperidone-induced insulin resistance hyperglycemia and dyslipidemia. Determination of the molecular action of metformin as well as the clinical studies to validate the therapeutic potential of metformin in attenuating risperidone-induced insulin resistance diabetes and dyslipidemia will be worth exploring in the near future.
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References


