Comparison of the efficacy of sitagliptin with pioglitazone on dexamethasone-induced hepatic steatosis, dyslipidemia, and hyperglycemia in albino rats

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INTRODUCTION

Two-third of the world’s population lives in the Asia-Pacific region where prevalence of diabetes has reached epidemic proportion. With China and India being the most populous nations on the globe, it is believed that over 150 million diabetes patients reside in the region with more than 95% being of type 2 diabetes mellitus (T2DM).1 The precise mechanism by which obesity leads to insulin resistance and to T2DM is not completely known but it may be related to several biochemical factors such as abnormalities in free fatty acids, adipokines, leptin, and other substances.2 Dysregulation of the secretion and/or activity of endogenous glucocorticoids are increasingly implicated in a number of common disorders that pose a growing clinical burden, such as obesity, Type 2 diabetes, the metabolic syndrome, hypertension and depression.3

Glucocorticoid action on target tissues is determined by the density of “nuclear” receptors and intracellular
metabolism by the two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) which catalyze interconversion of active cortisol and corticosterone with inert cortisone and 11-dehydrocorticosterone. 11β-HSD type 1, a predominant reductase in most intact cells, catalyzes the regeneration of active glucocorticoids, thus amplifying cellular action. 11β-HSD1 is widely expressed in liver, adipose tissue, muscle, pancreatic islets, adult brain, inflammatory cells, and gonads. 11β-HSD1 is selectively elevated in adipose tissue in obesity where it contributes to metabolic complications. Long-term upregulation of 11β-HSD1 in metabolically active tissues may follow prenatal “stress” hormone exposure and indicates a novel mechanism for fetal origins of adult obesity and the metabolic syndrome. An increased frequency of hypertension, central obesity, impaired glucose tolerance, diabetes, and hyperlipoproteinemia has been described in patients with subclinical Cushing’s syndrome.

Since the advent of glucocorticoid therapy for autoimmune disease in the 1940s, their widespread application has led to the concurrent therapy-limiting discovery of many adverse metabolic side effects. Unanticipated hyperglycemia associated with the initiation of glucocorticoids often leads to preventable hospital admissions, prolonged hospital stays, increased risks for infection, and reduced graft function in solid organ transplant recipients. Challenges in managing steroid-induced diabetes stem from wide fluctuations in post-prandial hyperglycemia and the lack of clearly defined treatment protocols.

Numerous studies have documented a strong relationship between hepatic steatosis and insulin resistance. In addition, there are several reports about the association of hepatic steatosis and glucocorticoids. Studies in mice have demonstrated that metformin reverses aminotransferase abnormalities, steatosis, and inflammation in mouse models of non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Many clinical trials have evaluated the useful effects of metformin on patients with NAFLD and NASH. A meta-analysis including five randomized controlled trials (RCT) concluded that metformin did not improve steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis in patients with NASH. A recent guideline indicates that metformin has no significant effect on liver histology and therefore, it is not recommended as a specific treatment for liver disease in adults with NASH.

A large meta-analysis that included 11 RCTs (862 participants, 38% diabetic) showed that Thiazolidinediones (TZDs) improve steatosis, hepatocellular ballooning and necroinflammation, delay fibrosis progression and ameliorate hepatic, muscle and adipose tissue insulin resistance with more consistent cardiovascular benefits with pioglitazone. Pioglitazone is associated with adverse events such as bladder cancer, bone loss, weight gain, painful swollen legs, and congestive heart failure. It would be a good choice to use TZDs for the treatment of NAFLD only in patients with T2DM who are also candidates for treatment with a TZD. In a study conducted in mice, exendin-4, a glucagon-like peptide-1 (GLP-1) agonist, reduced hepatic steatosis by improving insulin sensitivity. A recent meta-analysis including 4442 patients indicated that liraglutide decreased aminotransferase levels and that this effect was dose-dependent. It is concluded that controlled studies are needed to show the efficacy of GLP-1 analogs in NAFLD and NASH treatment. In rat models, dipeptidyl peptidase type 4 (DPP4) inhibitors improve hepatic steatosis by increasing insulin sensitivity and decreasing hepatic triglyceride levels. To date, there is no published controlled trial with these agents in humans.

**METHODS**

**Experimental animal**

Healthy adult rats of Wistar strain weighing around 240-270 g were used in the present study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature-controlled animal house with constant 12 hrs light/dark schedule. The animals were fed with standard rat pellet diet, and clean drinking water was made available ad libitum. All animal procedures have been approved, and prior permission from the Institutional Animal Ethical Committee (IAEC) was obtained as per prescribed guidelines (KSHEMA/IAEC/43/2011).

**Experimental methods**

A total of 24 rats were divided into four groups with six rats in each group. Body weight was checked for all groups on day 1, day 7, and day 12.

- First group received dexamethasone alone in a dose of 8 mg/kg intraperitoneally for 6 days to induce metabolic changes and considered as dexamethasone control.
- Second group received sitagliptin 100 mg/kg orally, 6 days before dexamethasone and 6 days during dexamethasone administration.
- Third group received pioglitazone 45 mg/kg orally, 6 days before dexamethasone and 6 days during dexamethasone administration.
- Fourth group did not receive any medication and was considered as normal control.

Rats were fasted overnight; blood was collected by retro-orbital sinus puncture for fasting blood sugar, lipid profile, and 2 hrs after a glucose load of 2 g/10 ml/kg IP (postprandial blood sugar). Rats were sacrificed by cervical dislocation. Liver was dissected out; liver weight, liver volume were measured. Livers were stored in 10% formalin and sent for histopathological analysis. The details of the study method are given in Table 1.
Statistical analysis

Data management was done in excel after cleaning and coding. Then, the data were transferred to SPSS package version 17.0 for analysis. The values presented as mean±standard deviation. Independent t-test was used to compare between two groups. ANOVA with Scheffe’s post-hoc test was done for multiple comparisons. A value of p=0.05 was considered statistically significant.

RESULTS

Effect of sitagliptin on blood sugar levels in rats

The blood sugar levels of four groups are presented in Table 2. An increase in blood sugar levels was seen in the dexamethasone group as compared to the normal group. A significant decrease in the fasting and postprandial blood glucose levels was observed in the sitagliptin- and pioglitazone-treated groups as compared to dexamethasone control group (p<0.01). The fasting blood glucose levels in the sitagliptin-treated group were comparable to pioglitazone group (p=0.342). A corresponding significant decrease in postprandial blood glucose levels was observed (p<0.01) in the pioglitazone group as compared with sitagliptin group (Table 2).

Effect of sitagliptin on lipids

The lipid profiles of four groups are presented in Table 2. An increase in total cholesterol, triglycerides, and decrease in high-density lipoprotein (HDL) was seen in the dexamethasone group as compared to the normal group. A significant decrease in the total cholesterol and triglyceride, and increase in HDL levels were observed in the sitagliptin- and pioglitazone-treated groups as compared to dexamethasone control group (p<0.01). The total cholesterol, triglyceride, and HDL levels in the sitagliptin-treated group were comparable to pioglitazone group (p=0.158, p=0.149, p=0.289) (Table 2).

Effect of sitagliptin on liver weight and liver volume

The liver weight and volume of four groups are presented in Table 3. An increase in the liver weight and volume was seen in the dexamethasone group as compared to the normal group. A significant decrease in the liver weight and volume was observed in the sitagliptin- and pioglitazone-treated groups as compared to dexamethasone control group (p<0.01). The liver weight and volume in the sitagliptin-treated group were comparable to pioglitazone group (p=0.158, p=0.149, p=0.289).

Table 1: Study protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1-12 (7 am)</th>
<th>Day 7-12 (7.30 am)</th>
<th>Day 11</th>
<th>Day 12 (8 am)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone group</td>
<td>10 ml/kg oral of 2% Gum Acacia</td>
<td>Dexa 8 mg/kg IP Overnight fast (from 4 pm)</td>
<td>Blood drawn for FBS, lipid profile and PPBS and rat was sacrificed, liver weight and volume measured and sent for histopathology</td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>10 ml/kg oral of 2% Gum Acacia</td>
<td>NS 2 ml/kg IP Overnight fast (from 4 pm)</td>
<td>Blood drawn for FBS, lipid profile, and PPBS and rat was sacrificed, liver weight and volume measured and sent for histopathology</td>
<td></td>
</tr>
<tr>
<td>Test drug group</td>
<td>100 mg/10 ml of 2% gum acacia per kg</td>
<td>Dexa 8 mg/kg IP Overnight fast (from 4 pm)</td>
<td>Blood drawn for FBS, lipid profile, and PPBS and rat was sacrificed, liver weight and volume measured and sent for histopathology</td>
<td></td>
</tr>
<tr>
<td>Standard drug group</td>
<td>45 mg/10 ml of 2% gum acacia per kg of pioz</td>
<td>Dexa 8 mg/kg IP Overnight fast (from 4 pm)</td>
<td>Blood drawn for FBS, lipid profile, and PPBS and rat was sacrificed, liver weight and volume measured and sent for histopathology</td>
<td></td>
</tr>
</tbody>
</table>

FBS: Fasting blood sugar, PPBS: Postprandial blood sugar

Table 2: Effect of sitagliptin on blood sugar levels and lipids in rats (n=6 per group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBS (mg/dl)</th>
<th>PPBS (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>90.33±2.81</td>
<td>111±3.36</td>
<td>89±5.31</td>
<td>69±1.71</td>
<td>39.67±2.64</td>
</tr>
<tr>
<td>Dexamethasone control</td>
<td>218±6.728</td>
<td>306.33±13.292</td>
<td>194.33±5.428</td>
<td>157.33±4.320</td>
<td>16±2.098</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>112±2.8288a</td>
<td>118.67±2.422</td>
<td>117.33±4.32a</td>
<td>88±1.789</td>
<td>38.67±2.944a</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>114±2.828ab</td>
<td>181±3.742a</td>
<td>122.67±4.32ad</td>
<td>89±3.742ad</td>
<td>37±1.789ad</td>
</tr>
</tbody>
</table>

Values are mean±SD, FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, HDL: High-density lipoproteins, SD: Standard deviation. *p<0.01 versus dexta control, **p=0.342 versus pioglitazone-treated group, ***p<0.01 versus sitagliptin-treated group, †p=0.158, ††p=0.149, †††p=0.289 versus pioglitazone-treated group
weight and liver volume was observed in the sitagliptin- and pioglitazone-treated groups as compared to dexamethasone control group (p<0.01). The liver weight and liver volume in the sitagliptin-treated group were comparable to pioglitazone group (p=0.378, p=0.095) (Table 3).

Effect of sitagliptin on body weight

The body weights of four groups are presented in Table 3. There was a weight loss seen in the dexamethasone group as compared to the normal group on day 12. A significant increase in the body weight was observed in the sitagliptin- and pioglitazone-treated groups as compared to dexamethasone control group (p<0.01). The body weight in the sitagliptin-treated group was comparable to pioglitazone group (p=0.280) (Table 3).

Histopathological observations

The Normal control group rats showed normal hepatocytes. The dexamethasone treated group showed increase in the size of hepatocytes, cytoplasm is vesicular to clear. Fat deposition was observed. The hepatocytes in rats treated with pioglitazone and sitagliptin were smaller in size and had reduced fat deposition compared to dexamethasone treated group (Figure 1).

DISCUSSION

Metformin and pioglitazone are the two insulin sensitizers used in current clinical practice.26 Considering their adverse effect profiles, metformin is more acceptable than pioglitazone. Even then, there is a situation in practice for the use of pioglitazone either as add-on or as a substitute to metformin.25 This is due to the difference in the mechanisms of action of metformin and pioglitazone. There is a need for an alternative insulin sensitizer whose efficacy is comparable to pioglitazone. Hence, it was decided to use pioglitazone as the standard.

Glucocorticoids are implicated in insulin resistance and T2DM. Large doses of glucocorticoids are used for therapeutic purposes, produce insulin resistance and diabetes mellitus for which insulin sensitizers like pioglitazone have been used commonly.27 However, pioglitazone has several adverse effects, which limits its use. GLP-1 RA is useful in preventing features of insulin resistance.28 Diet-induced adipose tissue inflammation and liver steatosis are prevented by DPP4 inhibition in diabetic mice.24 Sitagliptin is preferred to liraglutide, as it is effective orally.

Present study is done to compare the effects of sitagliptin with that of pioglitazone, on dexamethasone-induced hyperglycemia, dyslipidemia, and hepatic steatosis. Sitagliptin was as effective as pioglitazone in reducing the dyslipidemia, hepatic steatosis, and fasting hyperglycemia. However, it was not as effective in reducing blood glucose levels, 2 hrs after a glucose load. This may be because glucose load was given by intraperitoneal route. This is the limitation of this study. It is concluded that once-daily administration of sitagliptin might be useful in preventing the dyslipidemia, hepatic steatosis, and fasting hyperglycemia due to glucocorticoids excess.

ACKNOWLEDGMENTS

The authors acknowledge sincere thanks to the Department of Pharmacology (K.S Hegde Medical Academy), Nitte University Central Research Laboratory and the staff of animal house for the facilities granted for the research work.

Funding: Funded by Nitte University

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethical Committee of (K.S.Hegde Medical Academy), Nitte University, Mangalore, Karnataka, India

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