Effect of Pioglitazone and Levo-Carnitine on Plasma Glucose, Insulin Resistance and Serum Adiponectin in Type 2 Diabetic Mice

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Abstract

Background: To determine the effect of combined supplementation of pioglitazone and levo carnitine on plasma glucose, insulin resistance and serum adiponectin in T2DM mice.

Methods: In this randomized controlled trial 40 healthy BALB/c mice were divided into four groups (n=10 each). Mice were fed high fat diet for two weeks followed by intraperitoneal injection of streptozotocin to induce T2DM. Group I served as diabetic control. Group II was administered pioglitazone. Group III received levo carnitine and group IV was supplemented combined pioglitazone and levo carnitine. After six days of supplementation, terminal intra-cardiac blood extraction was carried out and samples were analyzed for plasma glucose, insulin resistance and serum adiponectin levels.

Results: Plasma glucose levels and insulin resistance significantly decreased in the combined supplementation group compared to the diabetic control. Serum adiponectin levels showed significant increase in combined supplemented group when compared with diabetic control and levo carnitine groups. The data also revealed a positive correlation between plasma glucose and insulin resistance and a negative correlation of these two parameters with serum adiponectin levels.

Conclusions: The combined supplementation of pioglitazone and levo carnitine significantly improved glycemic control, insulin resistance and serum adiponectin vis-à-vis individual supplementation in type 2 diabetic mice.

Key Words: Insulin resistance, Adiponectin, Pioglitazone, Levo carnitine, Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus is a complex heterogeneous group of metabolic disorders including hyperglycemia and impaired insulin action and/or insulin secretion. It causes dysfunction of multiple organs or tissues. Current theories of T2DM include a defect in insulin-mediated glucose uptake in muscles, dysfunction of the pancreatic beta-cells, disruption in secretory function of adipocytes, and impaired insulin action in liver. The etiology of human T2DM is multifactorial, with genetic background and physical inactivity as its two critical components. Obesity and weight gain are core contributors of T2DM and insulin resistance. T2DM is known to be associated with a range of many pathophysiological sequelae including hypertension, hyperlipidemia, atherosclerosis, etc. Increased insulin resistance occurs early in the natural history of T2DM but is compensated by increased secretion of insulin by β cells of pancreatic islets. When β cell failure arises, the hyperinsulinemia no longer can compensate for the insulin resistance and glucose homeostasis deteriorates.

Adipose tissue is now recognized as an important endocrine organ that secretes a number of biologically active proteins called “adipocytokines”. Adiponectin, is a novel adipocyte derived hormone also known as Acrp-30, Adipo-Q, apM-1 and GBP-28, which plays an important role in glucose metabolism and insulin resistance. Adiponectin gene is exclusively present abundantly in white adipose tissue and is a 244 amino acid, 30-KDa secreted protein with high structural homology to collagen VIII, X and complement C1q. In plasma, adiponectin circulates in high concentration, normally in the range of 3-30 µg/ml. Adiponectin exerts its effects through three cell membrane receptors, AdipoR-1, AdipoR-2 and T-cadherin. Obesity is inversely related to adiponectin making adiponectin a negative marker of metabolic syndrome. It is now well established that adiponectin plays an important role in T2DM, hypertension and dyslipidemias but the most significant role of adiponectin is its insulin sensitizing effect. In diabetic patients, the levels of adiponectin in blood are lower than normal, while higher levels of adiponectin minimizes the risk of developing T2DM. In liver and
skeletal muscle, adiponectin improves glucose utilization and stimulates fatty acid oxidation through a pathway that involves activation of AMP Kinase (AMPK) and acetyl-CoA carboxylase (ACC).\(^9\)

Pioglitazone is an oral antihyperglycemic agent belonging to a class thiazolidinediones (TZDs) and is widely used in the treatment of T2DM. Pioglitazone regulates glucose and lipid metabolism by activating the nuclear peroxisomes proliferator activated receptor gamma (PPAR-\(\gamma\)) which leads to increased transcription of different proteins thereby amplifying the post receptor action of insulin in the liver and peripheral tissues thus improving the glycemic control.\(^{10}\) Pioglitazone inhibits gluconeogenesis and increases the peripheral and splanchnic glucose uptake, thus augmenting the hepatic and peripheral insulin sensitivity.\(^{11}\) TZDs increases plasma adiponectin levels in animal models of obesity and diabetes, non-diabetic subjects and patients with T2DM. Thus it has been speculated that pioglitazone increases insulin sensitivity by mediating the hypoadiponectinemia in type2 diabetes.\(^{12}\)

Levo-carnitine is a quaternary ammonium compound that appears as a white crystalline, hygroscopic powder. Levo carnitine (\(\beta\)-hydroxy-\(\gamma\)-trimethylaminobutyrate) is a natural vitamin which is abundantly present in mammalian plasma and tissues. It is mainly distributed in skeletal and cardiac muscles. It is supplied to the body through dietary sources like meat and dairy products. It is also biosynthesized from lysine and methionine in the body. Its main function is to transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for \(\beta\)-oxidation.\(^{13}\) Levo carnitine infusion significantly improves insulin sensitivity and stimulates glucose uptake by skeletal muscles in healthy subjects and type 2 diabetic patients. Stimulation of glucose oxidation is manifested only in type 2 diabetic patients. There is a significant decrease in the plasma lactate levels after levo carnitine administration as well. These observations suggest levo carnitine-induced activation of the pyruvate dehydrogenase complex.\(^{14}\) Levo carnitine acts as an antioxidant, it is an intracellular superoxide scavenger that improves mitochondrial functions and reduces DNA damage. Acetyl-levo carnitine infusion acutely enhances insulin sensitivity in type 2 diabetics with insulin resistance.\(^{15}\)

The probable function of pioglitazone and levo carnitine in T2DM have been explored independently. However no interrelationship has so far been documented. Keeping this in view, the present study has been designed to evaluate the effects of combined supplementation of pioglitazone and levo carnitine on plasma glucose, insulin resistance and serum adiponectin in T2DM.

### Material and Methods

This study was undertaken at Department of Physiology, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. Blood glucose was measured by GOD-POD method. Enzyme linked immunosorbent assay (ELISA) was used to measure serum adiponectin. Serum triglycerides and serum HDL-C were estimated spectrophotometrically.

The study was conducted on 8-12 weeks old, 40 healthy BALB/c mice purchased from National Institute of Health, Islamabad. Average weight of mouse was 28.07 ± 0.1 g. Mice were divided into four equal groups. Free access to food and water was provided and the room was well ventilated with controlled temperature range of 20-22°C. 12 hours light and dark cycles were maintained.

Mice in all the four groups were fed on high fat diet ad libitum for 2 weeks after which 4 intra-peritoneal injections of streptozocin were given in the dose of 40mg/kg for 4 consecutive days.\(^{16}\) The mice continued to be fed on high fat diet during the 3rd week and no other supplementation was given during that period. At the end of the 4th week (after 10 days of STZ administration), mice were checked to confirm the development of T2DM by measuring the fasting plasma glucose (mg/dl). The blood glucose level > 252 mg/dl (14mmol/l) was taken as the cut off value for confirming diabetes mellitus.\(^{17}\) After establishing type 2 diabetes mellitus in all four groups, at the conclusion of 4th week, diabetic control group was supplemented with normal saline, pioglitazone group with pioglitazone (10 mg/kg body weight)\(^{18}\), levo carnitine group with levo carnitine (200 mg/kg body weight)\(^{19}\) and combined group with combined supplementation of pioglitazone and levo carnitine for 6 days. The terminal sample (1.5-2ml blood) was drawn at the end of the 5th week by a single intra cardiac puncture after 12 hours overnight fast. Data within the groups were analyzed by using ANOVA (one way analysis of variance) followed by Tukey’s HSD. \(p < 0.05\) was considered statistically significant.

### Results

The average weight of the mice was 28.07±0.10 grams at the start of the experiment. At the end of the 4th week of experiment (prior to the drug supplementation), the weight of mice in all the four
groups was found increased but had no statistical difference (p>0.05) amongst the groups (Table 1). Profound hyperglycaemia was developed in all the four groups having insignificant difference (p>0.05) amongst the groups (Table 1). Fasting plasma glucose of mice at the end of 5th week (after drug supplementation) showed a significant difference (p<0.001) amongst all the groups (Table 2). The combined supplementation revealed a highly significant reduction (p<0.001) in glycemic profile compared to the diabetic control (Table 3). Individual supplementation with pioglitazone and levo carnitine showed significant effect (p<0.001) when compared to the diabetic control. The glycemic control exerted by the combined supplementation of both the drugs was significantly pronounced (p<0.05) when compared with the levo carnitine group but not significant (p>0.05) when compared with pioglitazone group (Table 3).

Table 1: Plasma glucose, insulin resistance (IR) and serum adiponectin levels amongst the diabetic control, levo carnitine and combined supplementation groups by ANOVA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic control</th>
<th>Pioglitazone</th>
<th>Levo-carnitine</th>
<th>Combined</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>394.6 ± 39.66</td>
<td>395.2 ± 45.13</td>
<td>404.8 ± 43.15</td>
<td>390.4 ± 39.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Body weight (gm)</td>
<td>35.12 ± 2.40</td>
<td>35.07 ± 3.32</td>
<td>36.40 ± 2.12</td>
<td>35.62 ± 2.65</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

Table 2: Plasma glucose, insulin resistance (IR) and serum adiponectin levels amongst the diabetic control, pioglitazone, levo carnitine and combined supplementation groups by ANOVA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic control</th>
<th>Pioglitazone</th>
<th>Levo-carnitine</th>
<th>Combined</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>394.6 ± 39.66</td>
<td>252.50 ± 48.00</td>
<td>300.50 ± 33.54</td>
<td>256.30 ± 42.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>3.25 ± 0.95</td>
<td>1.48 ± 0.66</td>
<td>1.824 ± 0.60</td>
<td>1.25 ± 0.20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG: HDL ratio</td>
<td>7.83 ± 1.65</td>
<td>4.24 ± 0.85</td>
<td>8.35 ± 1.65</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Serum adiponectin (ng/dl)</td>
<td>3.97 ± 0.078</td>
<td>7.83 ± 1.76</td>
<td>4.24 ± 0.85</td>
<td>8.35 ± 1.65</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 3: Pearson correlation between plasma glucose, insulin resistance and serum adiponectin levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl) vs. Insulin resistance (IR)</td>
<td>r = 0.48</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl) vs. Serum adiponectin</td>
<td>r = 0.47</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Insulin resistance (IR) vs. Serum adiponectin</td>
<td>r = -0.33</td>
<td>p = 0.03</td>
</tr>
</tbody>
</table>

Insulin resistance (IR) was measured by TG/HDL ratio (which is a surrogate marker for assessing IR and its cut off value was ≥ 1.80).20 After the drug supplementation the IR showed significant difference (p<0.001) amongst all the four groups (Table 2). IR was higher than cut off value in the diabetic control group and decreased values were seen in pioglitazone, levo carnitine and combined groups (Table 2). The combined supplementation group showed significant reduction in IR (p<0.001) when compared to the diabetic control group. Individual supplementation of pioglitazone and levo carnitine also exhibited significant reduction in IR (p<0.001) when compared to the diabetic control group. The IR revealed reduction in combined supplementation but difference was not significant (p>0.05) when compared to the pioglitazone and levo carnitine groups (Table 3). The data showed significant difference (p<0.001) in serum adiponectin levels amongst the groups after the drug supplementation. Its level was reduced in diabetic control group and significantly increased (p<0.001) in drug supplemented groups (Table 2).
adiponectin. Whereas correlation between insulin resistance and serum adiponectin was negative, that manifested the insulin resistance in either groups and greater reduction in adiponectin level (Table 4).

**Discussion**

The mouse model of T2DM was prepared by high fat diet and intraperitoneal injection of streptozotocin (STZ) in the dose of 40 mg/kg body weight for 4 days because it closely resembled the gradual progression from obesity to insulin resistance and T2DM, which were the hallmarks of human phenotype. Confirmation of T2DM was done checking fasting blood glucose levels. The blood glucose level more than 252 mg/dl was taken as the cut off value for confirming T2DM. After the confirmation of the T2DM and prior to the drug supplementation, the glucose levels in all the groups ranged between 379-404 mg/dl. Pioglitazone supplementation resulted in statistically significant decrease in plasma glucose levels in pioglitazone group (252.50 ± 48.00 mg/dl) when compared with diabetic control group (413.70 ± 39.45 mg/dl). It is well documented that thiazolidinedione (TZD) such as pioglitazone decreases blood glucose in diabetic animal models and patients with T2DM. Pioglitazone being a PPAR-γ agonist increases the insulin sensitivity by increasing the transcription of insulin responsive genes. It enhances GLUT-4 and glucokinase activity, decreases inflammatory cytokines such as tumor necrosis factor alpha and resistin, inhibits gluconeogenesis and increases glucose uptake by the cells. In our study the plasma glucose level reduced to 67% by administration of 10 mg/kg pioglitazone intraperitoneally for 6 days. Similarly in an earlier study, the oral administration of 10 mg/kg body weight of pioglitazone for 7 days in HFD-fed/low dose STZ treated T2DM rat model (Sprague Dawley rats) presented with the reduction in plasma glucose level by 34.2%. The variation in level of effect between the two studies might be due to the difference in animal type and method of drug administration. Another study found that pioglitazone significantly improved glucose metabolism in HFD/STZ treated diabetic Wistar albino rats when 2.7 mg/kg/day pioglitazone was administered orally for 21 days which resulted in 32% reduction in blood glucose levels. These evidences indicate strong anti-hyperglycemic action of pioglitazone.

Levo carnitine in the dose of 200 mg/kg body weight of mice was administrated daily for 6 days to levo carnitine mice group and combined group. The same dose was used in a previous study for the same duration. Levo carnitine acts as a superoxide scavenger and a transporter of FFA that improves mitochondrial function by enhancing beta oxidation. It modulates the expression of glycolytic and gluconeogenic enzymes and thus regulates the hepatic glucose metabolism. In the present study, plasma glucose levels significantly reduced in levo carnitine group (330.50 ± 33.54 mg/dl) when compared to diabetic control group (413.70 ± 39.45 mg/dl). These results are consistent with the findings of another study in which administration of levo carnitine decreased blood glucose level in inbred strain of rats. Rahbar et al documented that fasting blood glucose levels in T2DM patients were significantly decreased by the oral intake of levo carnitine (1 gm/day) without any significant change in levels of HbA1c of these patients. In our study the most potent effect in lowering the plasma glucose level was observed in combined supplementation group (238.70 mg/dl). The combined administration of pioglitazone and levo carnitine achieved normoglycemia in diabetic mice indicating that the above combination of drugs will be more beneficial if given for longer duration to control the hyperglycemia.

Insulin resistance is the hallmark of early pathological disorder in T2DM. Plasma glucose and TG/HDL ratio in the HFD fed mice were increased. The combination of fasting hyperglycaemia and increased TG/HDL ratio in HFD fed mice were indicative of glucose intolerance and insulin resistance. In this study, significant insulin resistance developed in diabetic control group (TG/HDL ratio = 3.23). In another study, it was reported that the plasma triglyceride concentration, ratio of triglyceride to high density lipoprotein cholesterol concentration and insulin concentration were the most useful metabolic measures to identify the insulin resistant individuals.

Pioglitazone administration caused significant reduction in insulin resistance of pioglitazone group by bringing the TG/HDL ratio below 1.8 which was 1.48. This showed marked insulin sensitizing action of pioglitazone. Pioglitazone (PPARγ agonist) represents an important breakthrough in the therapy of insulin resistance. Its ability to increase adiponectin levels and insulin sensitivity suggests that there exists a link between adiponectin and insulin resistance. These findings are consistent with an earlier study that described the effect of pioglitazone which caused significant reduction in insulin resistance in individuals with T2DM by acting on the atherogenic lipoprotein profile.
Levo carnitine administration abolished the insulin resistance in carnitine group by bringing the TG/HDL ratio down to 1.824 and significant statistical difference was observed when compared to diabetic control group highlighting the insulin sensitizing action of levo carnitine. Levo carnitine causes increased transport of long chain fatty acids into the mitochondria for metabolism by its strong effect on the reaction catalysed by carnitine palmitoyl transferase-1 (CPT-1). High levels of levo carnitine cause reduction in cytosolic long chain acyl-CoA levels which might have improved the insulin signalling in diabetic mice of carnitine group. Insulin resistance improved significantly when obese diabetic transgenic mouse model was administered with levo carnitine orally (0.50% in diet i.e. 1 gm/kg/day) for three weeks.\(^{27}\) The significant improvement in IR (TG/HDL ratio = 1.25) in combined supplemented group showed a positive contribution of pioglitazone in normalizing the plasma glucose levels and levo carnitine in shunting the fatty acids into the mitochondria for oxidation, resulting in improved glycaemic control.

The serum adiponectin level was measured in all the four groups. Adiponectin is one of the most abundant secretory protein from adipose tissues in rodents and humans. A reduction in adiponectin level has been documented in obesity and T2DM in different studies and it has been suggested that adiponectin plays a protective role against insulin resistance. Weyer et al documented that the degree of hypo-adiponectinemia in people with obesity and T2DM is more closely related to the degree of insulin resistance and hyperinsulinemia. They also reported that adiponectin is the only adipose specific hormone which is exclusively produced in white adipose tissue and is negatively regulated in obesity.\(^{28}\) In our study the serum adiponectin level was found low in diabetic control mice (3.97 ng/dl). These findings are consistent with the results of another study on streptozotocin induced T2DM Sprague Dawley rats in which low adiponectin level was estimated in obese and diabetic groups (3.35 ng/dl in obese and 2.45 ng/dl in diabetic rats).\(^{29}\)

Thiazolidinedione (TZDs) increases plasma adiponectin level in animal models of obesity and diabetes and patients with T2DM. The improvement in insulin sensitivity in response to TZDs administration is associated with an increase in circulating adiponectin. Pioglitazone supplementation (10 mg/kg body weight) was given for six days to pioglitazone group and was found to cause significant increase (p<0.001) in serum adiponectin level (7.81 ng/dl) as compared to the diabetic control group (3.97 ng/dl). Similarly, it was demonstrated in an earlier experiment that insulin resistance and diabetes improved significantly in ob/ob mice in association with significant up-regulation of serum adiponectin levels with 14 days administration of 10 mg/kg body weight of pioglitazone. This strongly indicates that adiponectin is casually involved in the pioglitazone mediated amelioration of hepatic insulin resistance and diabetes.\(^{15}\) The finding of another investigation correspond to results of present study i.e. the administration of TZDs significantly increased the plasma adiponectin concentration in insulin resistant rodents (db/db mice).\(^{6}\) The effects of in vivo PPAR\(\gamma\) agonist (TZDs) treatment on plasma adiponectin levels in db/db mice and HFD/STZ treated mice found that mice treated with rosiglitazone at a dose of 10 mg/kg/day for 11 days (by daily oral gavage) showed three fold increase in mean plasma adiponectin level. Whereas, in our study, pioglitazone caused two fold increase in serum adiponectin level. The variance in degree of increase in adiponectin level may be due to the duration, type and method of drug administration.\(^{30}\)

Levo carnitine supplementation increased serum adiponectin level in levo carnitine group but difference was not significant when compared to the diabetic control group. An earlier study reported that chronic treatment with acetyl levo carnitine (oral supplementation) ameliorated the arterial hypertension, insulin resistance and hypoadiponectinemia in type-2 diabetic subjects at increased cardio vascular risk.\(^{16}\) A research conducted on obese women revealed that ingestion of levo carnitine 4 mg/kg body weight every other day for 8 weeks along with physical exercise resulted in a decreased leptin level and increased adiponectin level.\(^{31}\) In our study the strongest effect on adiponectin level was seen in combined supplemented group. The serum adiponectin level increased significantly (8.35 ng/dl) in this group as compared to the diabetic group (3.97 ng/dl). This study not only supported previous studies of individual efficacy of pioglitazone and levo carnitine but also suggested that increasing the number of mice and duration of the supplementation of both pioglitazone and levo carnitine may yield more promising prospects in this area.

**Conclusion**

Combined supplementation of pioglitazone and levo carnitine decreases plasma glucose, insulin resistance
and enhances the serum adiponectin levels that improves the insulin sensitivity in type 2 diabetic mice.

References