ORIGINAL ARTICLE

COMBINED EFFECT OF PIOGLITAZONE AND LEVO-CARNITINE ON SERUM LIPIDS AND ADIPONECTIN IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC MICE

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Background: A strong relationship exists between obesity and weight gain with type 2 diabetes mellitus (T2DM) because of the ability of obesity to cause insulin resistance. The objective of this study was to determine the effect of combined supplementation of pioglitazone and *levo* carnitine on serum lipids and adiponectin in streptozotocin induced T2DM mice. **Methods:** This randomized controlled trial was carried out on 40 healthy BALB/c mice, divided into four groups. Mice were fed high fat diet for two weeks followed by intraperitoneal injection of streptozotocin. Group I served as diabetic control; group II was administered pioglitazone; group III *levo* carnitine and group IV was supplemented pioglitazone and *levo* carnitine. After six days of supplementation, the blood samples were analysed to assess insulin resistance, serum adiponectin levels and lipid profile. **Results:** The serum adiponectin levels revealed significant increase in combined supplemented group as compared with both diabetic control and *levo* carnitine groups. An improved serum lipid profile was observed in combined supplemented group as compared to the diabetic control group. There was significant positive correlation between serum adiponectin levels with high density lipoprotein (HDL-C) levels and significant negative correlation between serum adiponectin with serum total cholesterol and low density lipoprotein (LDL-C) levels in diabetic control group. **Conclusions:** The combined supplementation of pioglitazone and *levo* carnitine increases serum adiponectin levels and ameliorates dyslipidemia better than individual administration of each supplement in type 2 diabetic mice.

Keywords: Adiponectin, Pioglitazone, Levo carnitine

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism resulting from defect in insulin secretion, insulin action, or both.[1] T2DM is a public health concern with multiple complications and increasing prevalence.[2] Insulin resistance is the primary feature in aetiology of T2DM. The genetic polymorphism of insulin receptor substrate-1 (IRS-1) leads to defective insulin signalling which plays a major role in insulin resistance.[3] Peroxisome proliferator activated receptor-gamma (PPAR-γ) is a transcription factor associated with regulation of fatty acid metabolism in skeletal muscle and adipose tissue, both being the principle sites of insulin resistance.[4]

The adipocytokines influence body adiposity, glucose homeostasis, inflammation and cardiovascular disease. Adiponectin, an adipokine also known as Acrp-30, Adipo-Q, apM-1 and GBP-28, plays an important role in glucose metabolism and insulin resistance. Adiponectin action on liver is insulin sensitizing and blood glucose lowering in diabetic animals by suppressing the gluconeogenesis. In liver and skeletal muscles, adiponectin improves glucose utilization and stimulates fatty acid oxidation through a pathway that involves activation of AMP Kinase and acetyl-CoA carboxylase.[5] One of the major contributing factors in T2DM is an abnormal lipid profile.[6]

Pioglitazone, an oral hypoglycemic, is a PPAR-γ agonist that increases the insulin sensitivity by increasing the transcription of insulin responsive genes which are involved in carbohydrate and lipid metabolism. Pioglitazone has been documented to decrease inflammatory cytokines, such as tumour necrosis factor-α (TNF-α) and interlenkin-6 (IL-6) while increasing the circulating level of adiponectin in the adipose tissues that activate PPAR-α receptors. It has been speculated that pioglitazone increases insulin sensitivity by ameliorating the hypoadiponectinemia in T2DM.[7] It has favourable effect on plasma lipids by improving the insulin sensitivity when used as mono-therapy or in combination resulting in greater decrease in triglycrides.[8]

Levo-carnitine is a natural vitamin, biosynthesized from lysine and methionine in the body and is found in meat and dairy products. *Levo*-carnitine is known as the biomarker to assess the function of mitochondria. It is the biologically active type of carnitine. Carnitine is an essential cofactor involved in the transport of long-chain fatty acids into the mitochondria for β-oxidation.[9]

Deficiency of carnitine and lack of suppression of lipolysis in diabetes leads to the impaired fatty acid oxidation resulting in elevated triglyceride, free fatty acid concentrations and excess accumulation of hepatic

fat. The carnitine exerts a substantial anti-oxidant action. It acts as an intracellular superoxide scavenger that improves mitochondrial function. These properties have been found to yield favourable effects on the function of peripheral nerve, diabetic heart, insulin resistance and vascular blood flow in the experimental diabetes. *L*-carnitine improved the fatty liver in high calorie diet/ streptozotocin induced type-2 diabetic mice by increasing fatty acid oxidation and decreasing *Levo*-carnitine/Acetyl *Levo*-carnitine ratio in liver.[10]

The interrelationship between pioglitazone and levocarnitine has not been explored so far. Keeping this in view, the present study was designed to evaluate the effect of combined supplementation of pioglitazone and *levo* carnitine on hypoadiponectinemia and dyslipidemia in T2DM.

MATERIAL AND METHODS

This Quasi Experimental study was undertaken at Department of Physiology, Army Medical College Rawalpindi from Sep 2012 to Aug 2013 after taking approval from Ethical Review Committee of the College. The study was conducted on 8–12 weeks old, 40 healthy BALB/c mice purchased from National Institute of Health, Islamabad. Average weight of the mouse was 28.07±0.1 g. Mice were divided into 4 equal groups. Free access to food and water was provided and the room was well ventilated with controlled temperature range of 20–22 °C. Twelve hours light and dark cycles were maintained. All mice were fed on high fat diet *ad libitum* for 2 weeks after which 4 intra-peritoneal injections of streptozotocin were given in the dose of 40 mg/Kg for 4 consecutive days.[11] 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), fasting glucose levels (mg/dl) were checked to confirm the development of T2DM. The blood glucose level >252 mg/dl (14 mmol/l) was taken as the cut-off value for confirming diabetes mellitus.[12] After establishing type 2 diabetes mellitus in all four groups, at the end of 4th week, diabetic control group was supplemented with normal saline, pioglitazone group with pioglitazone (10 mg/Kg body weight)[13], *levo* carnitine group with *levo* carnitine (200 mg/Kg body weight) and combined group with combined supplementation of pioglitazone and *levo* carnitine for 6 days. The terminal sample (1.5–2 ml blood) was drawn at the end of the 5th week by a single intra cardiac puncture after 12 hours overnight fast.

Data were analysed using SPSS-20. The arithmetic mean and standard deviation of all the variables were calculated. Data within the groups were analysed by using one-way ANOVA followed by Tukey's HSD test for pairwise comparison, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Serum adiponectin level was found reduced in diabetic control group whereas it significantly increased ($p<0.001$) in drug supplemented groups (Table-1). The data showed significant difference ($p<0.001$) in serum adiponectin levels amongst the groups after the drug supplementation. Serum adiponectin level significantly increased ($p<0.001$) in combined supplementation group when compared to the diabetic control group. Pioglitazone group also presented significant increase ($p<0.001$) in serum adiponectin level when compared with the diabetic control group. However, there was significant ($p<0.001$) increase in the serum adiponectin levels in pioglitazone supplemented group as compared to the *levo* carnitine group (Table-2).

Serum total cholesterol (TC) was found significantly reduced ($p<0.05$) in all the groups. Post Hoc test revealed that combined supplementation of pioglitazone and *levo* carnitine resulted in a significant reduction of serum total cholesterol levels ($p<0.05$) in the combined group compared to diabetic control group.

Diabetic control mice had higher levels of serum TG than the other three groups. ANOVA presents a significant difference ($p<0.05$) amongst all groups (Table-1). The comparison (Post Hoc) of the effect of pioglitazone and *levo* carnitine on serum TG levels revealed that combined supplementation of both the drugs significantly decreased the serum TG level ($p<0.05$) when compared to the control diabetic group. The efficacy of individual supplementation of both the drugs when compared to diabetic control was also significant ($p<0.05$) (Table-2).

Serum HDL levels were significantly increased in groups supplemented with drugs. The results of ANOVA (Table-1) described the statistical difference ($p<0.001$) amongst various groups. Pioglitazone significantly increased the serum HDL levels in type-2 diabetic mice in pioglitazone group as compared to the diabetic control ($p<0.05$). Similarly, *levo* carnitine also had significant effect ($p<0.05$) on increasing the HDL levels, either individually in *levo* carnitine group or in combination in combined group when compared with diabetic control.

Serum LDL and serum VLDL of type-2 diabetic mice amongst various groups after supplementation of drugs showed significant statistical difference ($p<0.05$) amongst all the groups (Table-1). Pioglitazone significantly ($p<0.05$) decreased the serum LDL and serum VLDL levels, when supplemented individually or in combination, as compared to the diabetic control whereas *levo* carnitine also yielded a significant LDL and VLDL lowering effect when used individually or in combination ($p<0.05$). *Levo* carnitine group showed significant difference ($p<0.05$) when compared with combined group (Table-2).

The Pearson's correlation coefficient between serum adiponectin and lipid profile revealed that serum adiponectin has significant positive association with serum HDL levels and significant negative association is seen with serum total cholesterol and serum LDL levels in diabetic control group (Table-3).

Table-1: Comparison of mean values of serum adiponectin and lipid levels between different study groups

Parameters	Diabetic control	Pioglitazone	*Levo* carnitine	Combined	*p*
Serum adiponectin (ηg/dl)	3.97±0.078	7.81±1.65	4.42±0.87	8.35±1.65	<0.001
Serum cholesterol (mg/dl)	180.80±30.07	154.30±23.65	162.10±19.84	140.30±15.61	0.003
Serum triglycerides (mg/dl)	198.90±32.89	138.90±25.12	156.30±34.74	116.80±17.09	<0.001
Serum HDL (mg/dl)	64.46±15.50	97.36±17.99	89.62±19.57	94.13±14.24	<0.001
Serum LDL (mg/dl)	76.55±35.49	29.15±11.46	41.33±8.62	22.80±11.41	<0.001
Serum VLDL (mg/dl)	39.78±6.57	27.78±5.02	31.14±6.85	23.36±3.41	<0.001

Note: Values have been expressed as Mean±SD

Table-2: Pairwise comparison of serum adiponectin and lipid levels along with statistical significance

Group Comparison	Serum adiponectin	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Pioglitazone vs diabetic control	<0.001	0.060	<0.001	0.001	<0.001	<0.001
L carnitine vs diabetic control	0.948	0.270	0.010	0.011	0.002	0.008
Pioglitazone vs *l* carnitine	0.001	0.870	0.524	0.739	0.530	0.549
Combined vs diabetic control	<0.001	0.002	<0.001	0.002	<0.001	<0.001
Combined vs pioglitazone	0.917	0.528	0.317	0.974	0.892	0.312
Combined vs *levo* carnitine	<0.001	0.164	0.018	0.933	0.181	0.019

Table-3: Pearson correlation of serum adiponectin with different parameters of lipid profile in diabetic control group

Parameters		*r*	*p*
Serum Adiponectin	TC	-0.935	<0.01**
	TG	0.666	0.774
	HDL-C	0.364	<0.01**
	LDL-C	-0.977	0.450
	VLDL-C	0.364	0.014*

*Significant at 0.05 level, **Significant at 0.01 level

DISCUSSION

Adiponectin plays a protective role against insulin resistance. It has been documented that there is a strong relationship between the degree of insulin resistance and hyperinsulinemia with the degree of hypo-adiponectinemia in people with obesity and T2DM and adiponectin is the only adipose specific hormone which is exclusively produced in white adipose tissue and is negatively regulated in obesity. In our study, low level of serum adiponectin was found in diabetic control mice (3.97 ηg/dl). These results are closely related with the findings of another study on streptozotocin induced T2DM Sprague Dawley rats in which low adiponectin level was measured in obese (3.35 ηg/dl) and diabetic group (2.45 ηg/dl).[14]

Pioglitazone supplementation alone or in combination was found to cause significant increase (p<0.001) in serum adiponectin level as compared to the diabetic control group. Similarly, it was demonstrated in an earlier experiment that administration of pioglitazone improved insulin resistance and diabetes significantly in ob/ob mice in association with significant up-regulation of serum adiponectin levels and this shows the key role of adiponectin in pioglitazone mediated amelioration of hepatic insulin resistance.[15]

In our study the strongest effect on adiponectin level was seen in combined supplemented group. The serum adiponectin level increased significantly in this group as compared to the diabetic group. It was reported in an earlier study that acetyl *Levo* carnitine (oral supplementation) when given for long duration, ameliorated the arterial hypertension, and hypoadiponectinemia in type-2 diabetic subjects. The effects of *L*-carnitine and physical activity on adipocytokines and lipid profile revealed that ingestion of *levo* carnitine 4 mg/Kg body weight every other day for 8 weeks in obese women resulted in increased adiponectin level and decreased leptin level.[16]

In present study, diabetic control mice showed high serum cholesterol, high serum triglycerides, low serum HDL, high levels of serum LDL and VLDL. In an earlier study, high fat diet/streptozotocin treated Sprague Dawley rats showed high levels of plasma total cholesterol and raised plasma triglycerides with frank hyperglycemia.[17]

In our study, the correlation between serum adiponectin and lipid profile in diabetic group revealed that serum adiponectin had significant positive association with serum HDL levels and a negative correlation was seen between serum adiponectin and serum TC and serum LDL levels. These results support the hypothesis that low levels of adiponectin in diabetes have strong relationship with diabetic dyslipidemia. It is documented in a study that adiponectin levels were strongly, positively, and independently related to HDL-C levels and hypoadiponectinemia may be a useful and more stable marker for monitoring the status of dyslipidemia in young women with polycystic ovarian syndrome (PCOS).[18] An earlier research showed the association of hypoadiponectinemia with dyslipidemia (low HDL-C and high TG) and uncontrolled glycemic status in type 2 diabetic subjects with and without coronary heart disease and reported that serum adiponectin was negatively associated with fasting

blood glucose and TG while positively associated with HDL-C in the studied population.[19]

In present study pioglitazone improved the abnormal lipid profile in pioglitazone group by increasing the serum HDL level significantly (p=0.001) while significant decrease (p<0.001) in serum triglyceride, serum LDL and VLDL levels as compared to diabetic control group. Pioglitazone has been shown in clinical trials to attenuate both blood glucose level and lipid profile when used as monotherapy or in combination with other antidiabetic agents. In monotherapy, pioglitazone has been associated with greater decrease in triglycerides and increase in HDL-C when compared with metformin and glibenclamide.[20] Pioglitazone affects glycemic control and atherogenic dyslipidemia in T2DM by improving the adiponectin levels since adiponectin levels in plasma are reduced in obese, diabetic or dyslipidemic subjects.[21] Another study reported that pioglitazone enhanced the adiponectin levels which contributed in improvement of HDL-C and TGs levels and HDL and LDL particle size.[22]

Levo-carnitine supplementation in present study revealed the significant (p<0.05) reduction in serum triglycerides, serum LDL and VLDL levels and significantly (p<0.05) increased serum HDL levels in *l*-carnitine group as compared to diabetic control group. *L*-carnitine causes increased insulin sensitivity leading to shifting of plasma lipids into adipose tissues along with increased consumption of the fatty acids via oxidative metabolism in mitochondria in type 2 diabetes. In another experiment, the effect of *l*-carnitine on lipid parameters of Swiss albino Wistar rats was studied. Their investigations showed that administration of *l*-carnitine (400 mg/day) orally for 4 weeks markedly decreased the serum total cholesterol, TG, LDL and VLDL levels but serum HDL was raised when *l*-carnitine was administered in high dose (800 mg/day).[23]

CONCLUSION

The combined supplementation of pioglitazone and *levo* carnitine enhances the serum adiponectin levels along with correction in diabetic dyslipidemia by improving the insulin sensitivity in type 2 diabetic mice.

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