REPORT

Liraglutide in diabetes mellitus: More facts and findings

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Abstract: Liraglutide exert favorable effects on most of the diabetes associated cardiovascular (CV) risk factors and this study was designed to further explore the benefits of liraglutide by observing its effect on plasma sialic acid (PSA) in diabetic rats. A total of 30 streptozotocin induced (50mg/Kg; i. p) diabetic rats were randomized into vehicle treated (1 ml/Kg s.c, twice daily) group I, liraglutide treated groups II and III (30µg/Kg and 150µg/Kg, twice daily respectively) and studied for 6 weeks. Liraglutide treated groups showed significant reductions in fructosamine levels ($p \leq 0.05$) from baseline. Between groups comparison revealed significant difference ($p \leq 0.05$) at the end point. Similarly, at week 6, liraglutide treated groups showed significantly low levels of PSA compared to baseline ($p < 0.03$ and $p < 0.005$ for group II and III respectively) and control group I ($p < 0.002$ and $p < 0.001$ for group II and III respectively). However, the difference was non-significant between groups II and III ($p < 0.09$). Other parameters including glucose tolerance, fasting plasma glucose (FPG), blood lipids, systolic blood pressure (SBP) and body weight also improved by liraglutide with the group III showing greater improvement. The study concludes that liraglutide produce favourable effects on PSA and may bea useful choice in protecting against diabetes associated CV complications.

Keywords: Liraglutide; GLP-1 analogue; plasma sialic acid; diabetic complications; rats.

INTRODUCTION

Achieving normoglycemia is the main target of diabetes treatment and is a measure of efficacy of hypoglycemic agents in one way but, the cluster of cardiovascular (CV) risk factors associated with diabetes also needs effective management. The use of combination therapy has greatly resolved the problem of glycemic control for a longer time period and some of these hypoglycaemic agents are also claimed to be antiatherogenic (UKPDS-33, 1998; Kahn *et al*., 2006). However, the occurrence of CV complications is still on the rise among diabetics and the number is increasing day by day (Lloyd-Jones *et al*., 2009). A newer hypoglycaemic agent, liraglutide is claimed to reduce blood glucose level and ameliorates most of the diabetes associated CV risk factors. It is glucagon-like peptide-1 (GLP-1) analogue and reduces HbA1-c, fasting plasma glucose (FPG), body weight and systolic blood pressure (SBP) more effectively than glimepiride monotherapy (Garber *et al*., 2009 and 2011). The evidence of the antiatherogenic properties of liraglutide diverted our attention to determine its effect on plasma PSA, which is a possible CV risk factor in diabetics and general population. The term sialic acid (SA) is used as a group name for derivatives ofneuraminic acid that occur mainly as part of terminal components of oligosaccharide chain present in glycoproteins and glycolipids. Increased levels of SA have been observed in various inflammatory disorders, malignancies as well as CV disease and diabetes (Dogan *et al*., 1992; Sillanaukee *et al*., 1999; Lindberg *et al*., 1992). In patients with established type 2 diabetes, a direct relationship between diabetic complications and high SA has been reported previously (Sillanaukee *et al*., 1999).

With the compelling evidence that liraglutide has more favourable effects on blood glucose and other CV risk factors in diabetic patients, the present study was carried out to observe the changes in PSA levels in experimentally induced diabetic rats treated with liraglutide.

MATERIALS AND METHODS

Animals

A total of 30 male albino rats (Animal house of National Institute of Health, Islamabad, Pakistan) aged 6-8 weeks and weighing 180-220g at baseline were randomized into three groups with ten rats in each group. They were fed on standard rat diet and water ad libitum. The rats were housed at a temperature of 25±2°C with a schedule of 12h light and 12h dark cycle, allowed acclimatizing for one

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week before carrying out any experimental work. Gandhara University Ethical Committee approved the research protocol of the present study before carrying out any experimental work.

Induction of experimental diabetes

The animals were fasted overnight and diabetes was induced by injecting streptozotocin (50mg/Kg; i p), freshly prepared in citrate buffer (3Mm) at pH 4.5 (Gajdosik *et al*., 1999). After a week, rats with stabilised diabetes having FPG values of >200mg/dl were considered diabetic and were included in the study. On day8 of streptozotocin injection, the treatment was started and noted as the day 1st of treatment.

Study design

Animals were randomized into 3 groups with the each group consisting of 10 rats. Group I received vehicle (1 ml/Kg s.c) twice daily and served as control. Group II was treated with liraglutide (30µg/Kg s.c) twice daily and group III was treated with liraglutide (150µg/Kg s.c) twice daily. The animals were dosed at 0815-0900 and 1500-1515h. Due to shorter half-life in rats, liraglutide was administered two times a day.

Sample collection

Blood for the determination of whole blood glucose concentration was collected intoheparinized glass tubes by puncture of the capillary vessels in the tail tip. For FPG and PSA determination, samples were centrifuged at 4ºC for 10 minutes and plasma was separated and stored at -20ºC until used. Part of the blood was collected in plain vials and allowed to clot for separation of serum, used for the estimation of fructosamine and lipid profile.

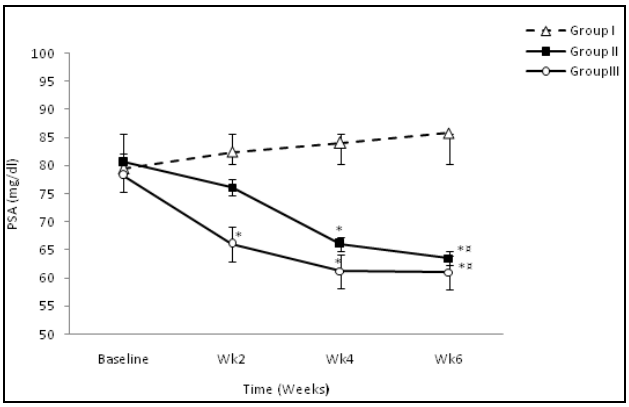
Biochemical/enzymatic estimation of different parameters

Sialic acid was determined by using a method proposed by Shamberger (1984). In brief, 0.5ml of serum was diluted with equal volume of distilled water and mixed well. Then the Ehrlich' s reagent (0.2ml) was added and the solution vortexed. After some time, white precipitate appeared and the tubes were then incubated in a water bath for 8 hours at 56°C with gentle agitation at 1, 3, 5 and 7 hours. After about 6 hours of incubation, the contents of the tube started turning blue. 3ml of normal saline was added to each tube and centrifuged at 2500 rpm for 15 minutes. The supernatant was then placed in a cuvette and read in a spectrophotometer at 525nm.

Oral glucose tolerance tests (OGTT) were performed in overnight fasted animals. Glucose solution (1g/Kg) was administered by gavage. Drug or vehicle was administered at regular time points and samples for the measurements of different parameters were drawn immediately prior to glucose load and at intervals thereafter. Fructosamine, FPG and blood lipids were analysed enzymatically by kits according to manufacturer's specification. Fructosamine, PSA, FPG and SBP were measured at baseline and then after every two weeks for 6 weeks. OGTTs were performed on days 21 and 41. Blood lipids and body weight were measured at baseline and at the end of the study.

STATISTICAL ANALYSIS

Results are expressed as means ±SD for 10 animals in each group. Differences of mean reduction in fructosamine, PSA, OGTT, FPG, SBP, blood lipids and body weight between groups were compared by ANNOVA. Differences with $p \leq 0.05$ were considered to be statistically significant.



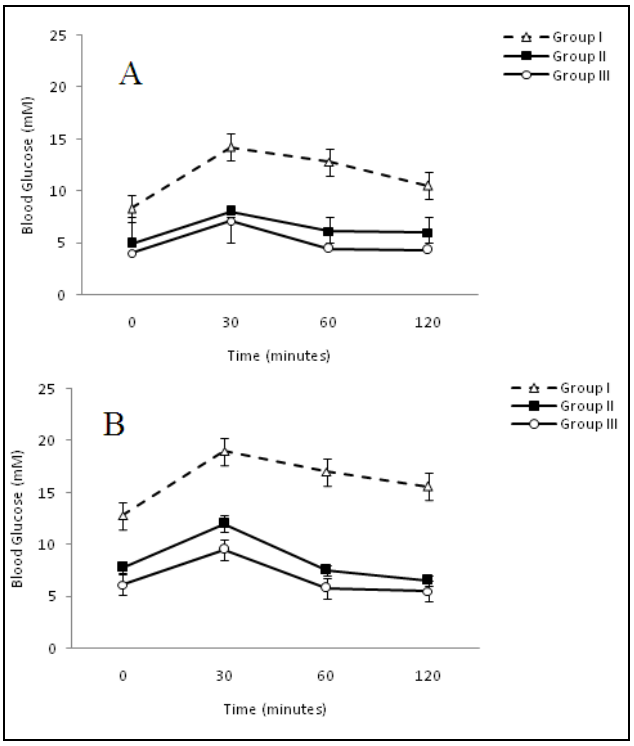
*Significant compared to base line (≤0.05)
*Significant compared to vehicle treated group I at Wk 6 (≤0.05)
Group I= vehicle treated; Group II= Liraglutide (300µg/day); Group III= Liraglutide (150 µg/day)

Fig. 1: Changes in PSA levels over time

RESULTS

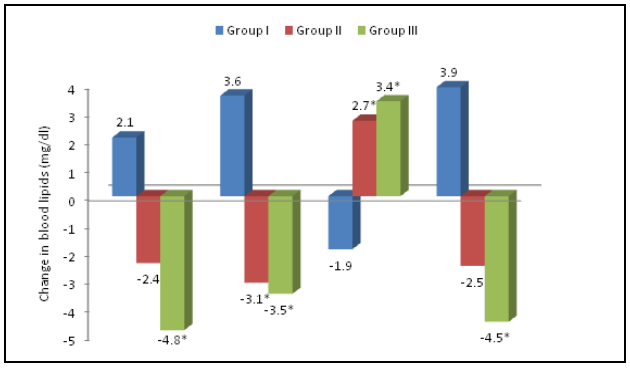
Compared to base line, there were significant reductions ($p \leq 0.05$) in fructosamine levels in liraglutide treated group II (30µg/Kg) and group III (150µg/Kg) at week 6. Differences were seen even at the 2nd week of treatment and continued till the end of the study period. At week 6, between groups comparison revealed significantly low levels ($p \leq 0.05$) of fructosamine in liraglutide treated groups than the vehicle treated group (table1). PSA also showed a trend towards decreased levels in liraglutide treated groups. The temporal pattern of these reductions were however different among the groups. In-group II, the level decreased from 80.6±8.5mg/dl to 76.1±6.3mg/dl ($p<0.47$) between week 0-2-, further decreased to 65.9±8.1mg/dl ($p<0.05$) between week 3-4 and finally reached to 63.8±7.4mg/dl ($p<0.03$) between week 5-6. In group III, the level dropped from 78.9±9.4mg/dl to 66.0±7.1mg/dl ($p<0.01$) between week 0-2, further decreased to 61.3±6.9 mg/dl ($p<0.005$) between week 3-4 and then remained almost stable (61.2±6.4mg/dl, $p<0.005$) between week 5-6. Comparison of PSA levels of

group II and group III at week 6 was not significantly different (p<0.09). However, when liraglutide treated groups were compared to vehicle treated control group at week 6, statistically significant differences (p<0.002 and p<0.001 for group II and group III respectively) were observed (fig. 1).



OGTTs were performed at days 21 (A) and 41 (B) of liraglutide treatment. Overnight fasted rats received glucose (1g/Kg) at time 0 and blood glucose was measured basally and after the glucose challenge.
A. ANNOVA revealed significant difference between the groups (p<0.005)
B. ANNOVA revealed significant difference between the groups (p<0.001)

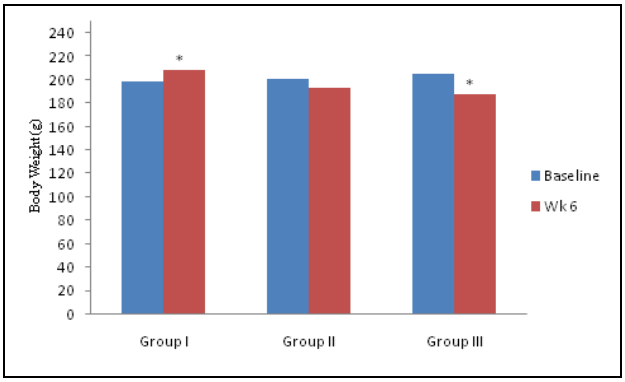
Fig. 2: Oral glucose tolerance test (OGTT)



*Significant compared to baseline (≤0.05) TC=Total Cholesterol; LDL-c= Low density lipoprotein-cholesterol; HDL-c= High density lipoprotein-cholesterol; Tg= Triglyceride

Fig. 3: Changes in blood lipids

Fig. 2A & B shows the 1st and 2nd OGTT carried out on days 14 and day 27 of treatment respectively. Glucose excursions were significantly reduced both in group II and group III compared to group I (p<0.005 and p<0.001 respectively). Liraglutide treatment was also associated with significant reductions (p≤0.05) in FPG levels. Compared to base line, statistically significant decreases were observed starting from the 2nd week of treatment till the end point with the group III showing greater reduction than the group II (table 1).



*Significant compared to baseline (≤0.05)

Fig. 4: Changes in body weight

The changes occurred in blood lipid levels by liraglutide were -2.4 and -4.8 mg/dl in total cholesterol (p<0.08 and p<0.01 respectively), -3.1 and -3.5 mg/dl in low density lipoprotein cholesterol (p<0.03 and p<0.01respectively), +2.7 and +3.4 mg/dl in high density lipoprotein cholesterol (p<0.01 and p<0.004 respectively) and −2.5 and -4.5 mg/dl in triglyceride (p<0.05 and p<0.01 respectively) in group II and group III respectively (fig. 3). Furthermore, liraglutide produced significant improvement ((p≤0.05) in systolic blood pressure (SBP) both at low and high doses however, the decrease in body weight was significant (p≤0.05) only in group III (table1, fig. 4).

DISCUSSION

The demand for hypoglycemic agents with complementary mechanisms of actions capable of addressing and/or modulating multiple CV risk factors associated with diabetes is due to the fact that diabetes itself is a CV disease (Scott *et al*., 1999). Different treatment strategies are continuously being employed in order to reduce and /or in part delay the incidence of CV complications in diabetes, but unfortunately no one has achieved such a high success rate to be declared as an ideal treatment. Even with the optimal management of blood glucose, deaths due to CV complications are still on the rise among diabetics (Nathan *et al*., 2005). Therefore, more attention is needed to be focused on the effect of hypoglycaemic agents on other possible CV risk factors such as sialic acid, which is believed to play a role in the

Table 1: Effect of liraglutide on fructosamine, FPG and SBP of diabetic rats

Fructosamine (µ mol/L)	Baseline	Wk. 2	Wk. 4	Wk. 6
Group I	311.4±18.2	323.1±13.5	315.8±20.6	326.7±118.2*
Group II	318.0±14.3	298.1±10.2*	291.0±8.8*	288.9±10.1*¤
Group III	307.9±13.5	292.5±8.1*	286.0±13.7*	283.4±9.9*¤
FPG (mM/L)				
Group I	12.9±1.4	13.5±1.8	13.7±1.2*	14.1±1.3*
Group II	12.5±1.1	10.3±0.9*	9±1.3*	7.1±1.2*¤
Group III	12.2±1.9	9.6±1.1*	7.6±1.5*	6.5±1.5*¤
SBP (mmHg)				
Group I	128.7±4.2	131.8±4.6	130±6.9	132.8±5.5
Group II	130±4.3	129.6±2.8	129.5±3.1	129.2±2.9¤
Group III	128.5±3.1	127.9±4.3	126.6±3.9*	126.1±2.8*¤

Data are mean ±SD of 10 rats in each group
FPG= Fasting Plasma Glucose; SBP= Systolic blood pressure
Group I=vehicle treated; Group II=liraglutide (30µg/Kg); Group III= liraglutide (150µg/Kg)
Wk. Week; FPG= Fasting Plasma Glucose; SBP= Systolic Blood Pressure
*Significant compared to base line (≤0.05)
*Significant compared to vehicle treated group at Wk 6(≤0.05)

pathogenesis of diabetic complications (Lindberg *et al*., 1991; Knuiman *et al*., 2004).

Results of the present study indicate that liraglutide PSA lowering properties along with exerting hypoglycemic effect. A significant decline in PSA indicates that liraglutide may bring about specific changes in metabolic processes that could be associated with regression of diabetic complications. Earlier, we have shown that some of the hypoglycemic agents ameliorate PSA level in diabetics to a variable extent probably by manipulating the aggressive hexosamine pathway and this is in agreement with the results of the present study (Rahman *et al*., 2010). However, the previous studies were conducted for a longer time period (1-3 years) and a significant difference in PSA levels were observed only at the end points. A more rapid decrease by liraglutide may be the result of its beneficial effects beyond glycemic control. It has been shown previously that liraglutide treatment is associated with a reduction in the levels of certain acute phase proteins that function as CV biomarkers and are associated with higher risk of CVD (Courreges *et al*., 2008). Elevated levels of acute phase proteins in diabetes contribute to high PSA, which in turn reflects the existence or the activity of ongoing atherosclerotic process. At a larger dose (150µg/Kg), liraglutide showed still greater reduction in PSA throughout the study period and the level remained almost stable between weeks 4 to 6. We do not have a solid reason for this but it may be possible that there is no further decrease in diabetics. Moreover, liraglutide treatment was associated with improvement in blood lipids, body weight and systolic blood pressure and these results are well supported by previous findings (Vilsboll *et al*., 2012; Plutzky *et al*., 2009). A positive association between sialic acid (SA) and blood lipids has been found previously (Wakabayashi *et al*., 1992). There are reports of high SA levels in type 2 diabetic patients treated with oral hypoglycemic agents than on diet control only (Crook *et al*., 1993). However, all of the patients receiving oral agents had retinopathy in which sialic acid levels were found to be elevated. A significant decrease in SA levels has also been observed in growth hormone deficient adults, after three months growth hormone replacement therapy (Christ *et al*., 1999). Similarly, changes in SA in Dalton's lymphoma bearing mice after cyclophosphamide and cisplatin treatment indicate that SA levels are affected by drug treatment (Nico and Prasad, 2002) and these observations well support the findings of the present study.

CONCLUSION

Our findings provide another evidence of the antiatherogenic effects of liraglutide by exerting ameliorative effect on PSA in diabetes mellitus. We conclude that liraglutide may be helpful in protecting and/or at least delaying the atherosclerotic process in diabetics.

REFERENCES

Christ ER, Cummings MH, Lumb PJ, Crook MA, Sonksen PH and Russell-Jones DL (1999). Growth hormone replacement therapy reduces serum sialic acid concentration in adults with growth hormone deficiency: A double blind placebo controlled study. *Clin. Endocrinol.*, **51**: 173-179.

Courreges JP, Vilsboll T, Zdravkovic M, Thi TL, Krarup T and Schmitz TO *et al* (2008). Beneficial effects of

once-daily liraglutide, a human glucagon-like peptide-1 analogue, on cardiovascular risk biomarkers in patients with type 2 diabetes. *Diabet. Med.*, **25**: 1129-1131.

Crook MA, Haq M, Haq S and Tutt P (1993). Serum sialic acid and lipid associated sialic acid in normal individuals and patients with myocardial infarction, and their relationship to acute phase proteins. *Ann. Clin. Biochem.*, **30**: 383-386.

Dogan H, Pasaoglu H, Ekinciler OF and Tatlisen N (1992). A comparative study of total protein, total and lipid associated serum sialic acid levels in patients with Behcet's disease and control groups. *Acta. Ophthalmol.*, **70**: 790-794.

Gajdosik A, Gajdosikova A, Stefek M, Navarova J and Hozova R (1999). Streptozotocin induced experimental diabetes in male Wister rats. *Gen. Physiol. Biophys.*, **18**: 54-62.

Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez PH and Olvera AI *et al*. (2009). Liraglutide versus glimepiridemonotherapy for type 2 diabetes (LEAD-3 Mono): A randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet*, **373**: 473-481.

Garber A, Henry, R, Ratner, R, Hale P, Chang CT and Bode B (2011). Liraglutide, a once-daily humanglucagon-like peptide 1 analogue, provides sustained improvements in glycaemiccontrol and weight for 2 years as monotherapy compared with glimepiride in patients with type 2 diabetes. *Diabetes Obes Metab,* **13**: 348-356.

Kahn SE, Haffner SM, Heise MA, William H, Herman WH, Holman RR and Jones NP *et al*. (2006). Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *New Eng. J. Med.*, **355**: 2427-2443.

Knuiman MW, Watts GF and Divitini ML (2004). Is sialic acid an independent risk factor for cardiovascular disease? A 17 year follow-up study in Buss Elton, Western Australia. *Ann. Epidemiol.*, **14**: 627-632.

Lindberg G, Eklund GA, Gulberg B and Rastam L (1991). Serum sialic acid concentration and cardiovascular mortality. *BMJ.*, **302**: 143-146.

Lindberg G, Rastam L, Gullberg B and Eklund GA (1992). Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow-up. *Int. J. Epidemiol.*, **21**: 253-257.

Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB and Flegal K *et al*. (2009). Heart disease and stroke statistics-2009 update: A report from the American heart association statistics committee and stroke statistics subcommittee. *Circulation,* **119**: e21-e181.

Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM and Orchard TJ *et al*. (2005). Intensive diabetes treatment and cardiovascular disease in patients with ty pe 1 diabetes. *New Eng. J. Med.*, **353**: 2643-2653.

Nico BM and Prasad SB (2002). Sialic acid changes in Dalton's lymphoma bearing mice after cyclophosphamide and cisplatin treatment. *Br. J. Med. Biol. Res.*, **5**: 549-553.

Plutzky J, Garber A, Toft AD and Poulter NR (2009). Meta-analysis demonstrates thatliraglutide, a once-daily human GLP-1 analogue, significantly reduces lipids and other markers of cardiovascular risk in type 2 diabetes. *Diabetologia,* **52**: S299-S300.

Rahman IU, Malik SA, Bashir M, Khan R and Khan MI (2010). Serum sialic acid changes in type 2 diabetic patients on metformin or rosiglitazone treatment. *J. Clin. Pharm. Ther.,* **35**: 685-690.

Scott MG, Ivor JB, Gregory LB, Alan C, Robert HE and Barbara VH *et al*. (1999). Diabetes and cardiovascular Disease. *Circulation,* **100**: 1134-1146.

Shamberger RJ (1984). Serum sialic acid in normal and cancer patient. *Clin. Chem. Biochem.,* **22**: 6451.

Sillanaukee P, Ponnio M and Jaaskelainen IP (1999). Occurrence of sialic acids in healthy humans and different disorders. *Eur. J. Clin. Invest*, **29**: 413-425.

UK Prospective Diabetes Study (UKPDS-33) (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications inpatients with type 2 diabetes. *Lancet,* **352**: 837-853.

Vilsboll T, Christensen M, Junker AE, Knop FK, Gluud LL *et al*. (2012). Effects of glucagon-likepeptide receptor agonists on weight loss: Systematic review andmeta-analyses of randomized controlled trials. *BMJ*, **344**: 1-11.

Wakabayashi I, Sakameto K, Yoshimoto S and Masui S (1992). Relation of serum sialic acid to lipid concentration. *BMJ.*, **305**: 562-563.