DIPEPTIDYL PEPTIDASE – IV (DPP-IV) INHIBITORY ACTIVITY OF METFORMIN

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Summary

Dipeptidyl peptidase IV is plasma membrane glycoprotein exopeptidase, inhibition of DPP-IV activity could be a useful strategy to enhance the activity of GLP-1. GLP-1 is an incretin released from L cells in the intestine after oral ingestion of nutrients. GLP-1 (7–37) is actively being evaluated as a therapy for diabetes mellitus. Metformin is an oral biguanide agent used for the treatment of type 2 diabetes. Male Wistar rats were used for the study. Serum glucose and DPP-IV levels (0 day) were compared with post treatment values on day 15th. Lipid profiles were determined after 15th day drug treatment. In alloxan induced diabetic rats, acute administration of metformin significantly reduced the serum glucose and DPP-IV levels from 0 day to until the end of the experiment. Similarly there was a significance influence on lipid profile on acute administration of metformin drug treated in alloxan induced diabetic rats. DPP-IV levels and lipid profiles were reduced (except HDL) in metformin drug treated diabetic rats. Therefore, metformin used a DPP-IV inhibitor and anti-dyslipidimic effect. The present investigation thus encourages the development of new metformin analogues with better DPP-IV activity.

Key Words: Dipeptidyl peptidase-IV(DPP-IV), glucagon like peptide-1 (GLP-1), metformin,

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Introduction

Type 2 diabetes is a heterogeneous clinical syndrome characterised by elevated blood glucose levels due to insulin resistance [1]. GLP-1 is an incretin released from L cells in the intestine after oral ingestion of nutrients. It has multiple actions, including stimulation of inhibition of glucagon secretion, increase of glycogen synthase activity, and slowing of gastric emptying, in addition to promotion of satiety and inhibition of food intake [2]. It also reduces food intake and body weight gain in subjects treated with metformin which might be related to GLP-1 increase [3]. GLP-1(7-37) is rapidly metabolized by the enzyme dipeptidylpeptidase-IV (DPP-IV) to release the major degradation fragment GLP-1 (9-37) [4]. The resulting substance has circulatory half-life of only 1 to 2 min. Dipeptidyl peptidase IV is plasma membrane glycoprotein exopeptidase that belongs to the prolyl oligopeptidase family [5]. Thus, inhibition of DPP-IV activity could be a useful strategy to enhance the activity of GLP-1. GLP-1 (7–37) is actively being evaluated as a therapy for diabetes mellitus. Its exogenous administration to nondiabetic and type 2 diabetic subjects results in lowering blood glucose [6]. Metformin is an oral biguanide agent used for the treatment of type 2 diabetes. It lowers plasma glucose through reduction of hepatic glucose production [7] and alleviation of insulin resistance [8]. It has been shown to decrease triglyceride concentrations, decrease free fatty acids, decrease total cholesterol and increase HDL cholesterol [9]. There are few reports on DPP-IV inhibitory activity of metformin. Therefore, we attempted in this study to investigate the effects of metformin on DPP-IV inhibitory activity and lipid profiles for developing its analogs with better anti diabetic activity.

Methods

Materials

Dipeptidyl peptidases-IV (enzyme) and glycine - proline p- nitroanilide (substrate) were purchased from Sigma, St. Louis, MO, USA. Tris HCL and phosphate buffers (pH7.6) were purchased from E. Merck Ltd, Mumbai, India. Metformin pure substance was a kind gift from Cadila pharmaceuticals Ltd, Ahmedabad, India. Vildagliptin pure substance was a kind gift from Novartis health care Ltd, Mumbai, India

Animals and drug treatment

Male Wistar rats, weighing between 180-200g were obtained from Mahaveer Enterprises, Hyderabad. The selected animals were housed five per each of acrylic cages at 25°C, 45-55% humidity and 12/12 h light/dark under controlled environment. Rats were fed with standard laboratory diet and water was given *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use. The experiment protocol was approval from the institutional animal ethical committee. Animals were divided into four groups containing six animals each. Blood sample was drawn by making small incision on the tail vein from all fasted rats (Day 0). These rats were made diabetic using single injection of alloxan monohydrate (130 mg/kg, *i.p*) in normal saline solution. Animals were treated with 10% dextrose orally to combat the early phase of hypoglycaemia.

The induction of diabetes mellitus was confirmed after 48 hours of alloxan treatment by estimation of elevated fasting blood glucose (FBG) level. Only those rats with blood glucose level ≥ 200 mg/dl were included in the study (Day 0). Group I served as normal. Group II was treated with alloxan only and group III was treated with DPP-IV inhibitor vildagliptin (30 mg/kg, *p.o*), while group IV received metformin (300 mg/kg, *p.o*) for 14 days. Treatment with drugs was started on the 2nd day of the alloxan treatment (i.e. Day 1) and was continued for 2 weeks. All the drugs were given orally as a single dose in the morning (10.00A.M). Blood glucose was measured before starting the treatment (Day 0) and weekly thereafter up to the end of the treatment period and estimated fasting blood glucose by glucose-oxidase-peroxidase (GOD-POD) method [10]. Different concentrations of DPP-IV (10, 20, 30, 40,50,60,70 and 80 U/L) in rat serum were prepared for calibration curve. DPP-IV activity was measured by spectrophotometry [11] and lipid profile includes total cholesterol (TCh), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high-density lipoprotein (HDL) were estimated after the completion of the treatment period (i.e. at the end of the 2nd week) by enzymatic method using reagent kit[12]. Serum glucose and DPP-IV levels (Day 0) were compared with post treatment values on day 15th.

Statistical analysis

All variables are expressed as mean ± SD. Group differences were compared using ANOVA followed by Newman Keuls *post hoc* test. For all analyses, a P value < 0.05 was considered to be statistically significant. The statistical package Graph-Pad Prism, version 4 for Windows (San Diego, CA, USA) was used in the analyses.

Results

Upon statistical analysis, (ANOVA) compared groups (control, alloxan induced, vildagliptin and metformin) for 14 days it was given results that increased blood glucose and DPP-IV levels in alloxan induced group. In alloxan induced diabetic rats, acute administration of metformin significantly reduced the serum glucose and DPP-IV levels from 0 day to till the end of the experiment. DPP-IV levels in metformin drug treated group showed significantly lower (35.71± 4.82 U/l) than in alloxan induced diabetic rats (56.12 ± 8.87 U/l).

In alloxanised animals showed increased total cholesterol (TC), triglycerides (TG), LDL, (Low density lipoprotein) and VLDL (Very low density lipoprotein) except HDL (High density lipoprotein) while the metformin treated group showed increased HDL, Which were stastically significant (P<0.05).

Table 1

Effect of serum DPP-IV and glucose level of diabetic rats at 0 and 15th day intervals.

	Blood Glucose (mg/dl)		DPP-IV (U/L)	
Groups	0 Day	15th Day	0 Day	15th Day
Normal	76.45 ± 5.60	83 ± 2.56	14.95 ±1.21	16.57 ± 1.31
Alloxan	285 ± 12.24	365 ± 14.60	62.03 ± 4.12	60.76 ± 4.63
Metformin	278 ± 18.61	128 ± 6.55	58.46 ± 2.84	35.03 ± 3.76
Vildagliptin	274 ± 18.78	82 ± 6.16	61.16 ± 6.66	24.67 ± 3.39

Data values were expressed as mean ± SD (n= 6).

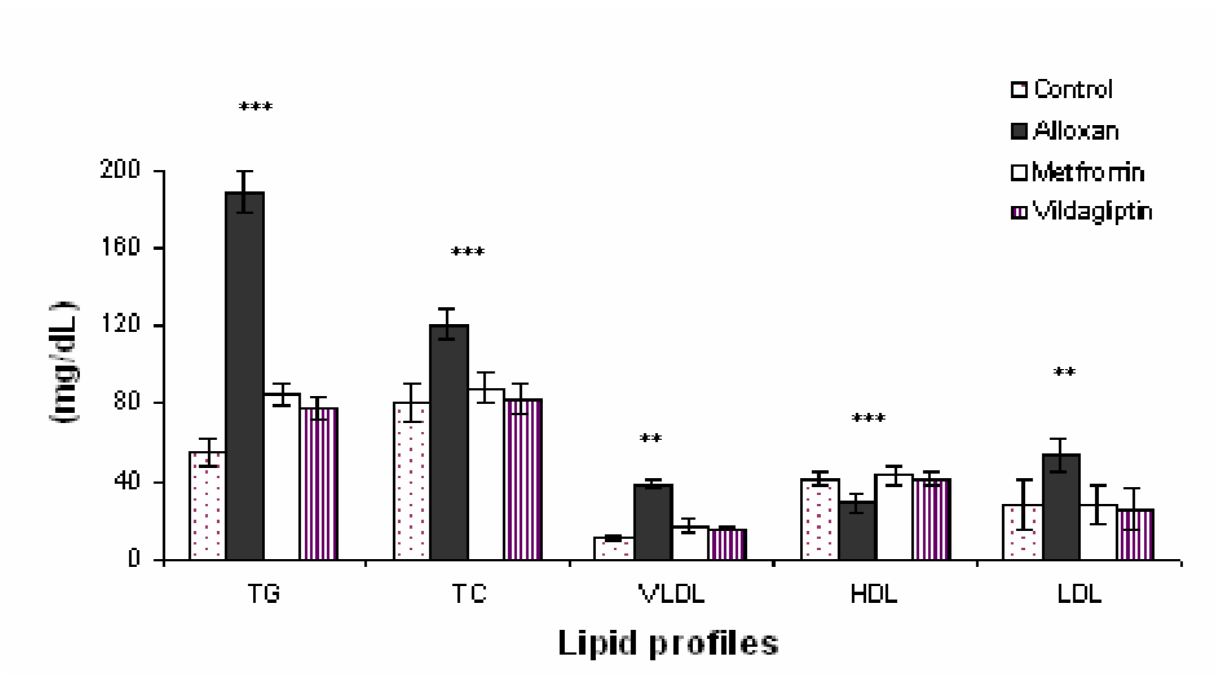

Figure 1. Serum lipid profiles in different group of rats.

Data values are expressed as mean ± SD (n=6). ** $p < 0.01$, *** $p < 0.001$

Discussion

Vildagliptin is a selective, reversible, competitive inhibitor of dipeptidyl peptidase IV enzyme[13]. DPP-IV enzyme that contributes to the in activation of the hormone glucagon like peptide-1 (GLP-1). Inhibition of DPP-IV results in increased levels of active GLP-1. GLP-1 is a hormone secreted by the intestines from L in cells response to food intake[14], and stimulates insulin production. The 12 week treatment with vildagliptin produced a placebo-subtracted reduction in HbA1c of 0.7% in patients with baseline HbA1c levels of 7.7% while on a stable dosage of metformin. After 52 weeks, the between-group difference in HbA1c was 1.1%, reflecting deterioration of glycemic control in placebo plus metformin treated patients and a stable HbA1c of 7.1% from week 12 to week 52 in patients treated with vildagliptin plus metformin. The mechanism by which vildagliptin improved glycemic control in these metformin-treated patients was not directly addressed; however, it is attributable to inhibition of DPP-IV and resultant increases of circulating levels of the intact, biologically active incretins. Although plasma DPP-IV activity, GLP-1, and GIP were not measured , earlier studies have shown that vildagliptin profoundly suppresses DPP-IV activity and increases plasma levels of intact GLP-1[15]. In our present investigation, metformin significantly reduces DPP-IV levels in metformin treated diabetic rats than alloxan induced diabetic rats.

Recently, it has been demonstrated that oral metformin therapy effectively inhibits DPP-IV activity in patients with type 2 diabetes and it may contribute to glycemic control by reducing the degradation of enteroinsular hormones secreted following feeding [16]. A recent study demonstrated that 14 -day metformin treatment led to higher active levels of GLP-1. It also inhibited the degradation of GLP-1(7-36) amide to GLP-1(9-36) amide in pooled human plasma and it was concluded that inhibition of GLP-1 degradation by metformin would explain the higher levels of active GLP-1 observed (Mannucci *et al.,* 2001). In another study, it was observed that metformin treatment increased active levels of GLP-1 in rats. It was concluded that it was due to metformin increases GLP-1 secretion and does not directly inhibit DPP- IV [17]. Metformin was reported to lowers basal insulin but increases the postprandial insulin response. The present study result reveals that metformin might inhibit DPP-IV leads to increased active GLP-1 levels and improves the glucose tolerence.

Metformin was found to enhance the anorexic and fat-losing effects of leptin in standard chow rats and to restore leptin sensitivity in high-fat–fed obese rats with leptin resistance [18]. Metformin produces beneficial effects on the lipid profile mainly by correcting abnormal glucose metabolism [19]. It also produces moderate reduction in the triglyceride levels as a result of decreased hepatic synthesis of very low-density lipoprotein (VLDL) and decreases total cholesterol levels through a decrease in LDL-C[20] and there was a trend toward increased HDL-C [21]. In our present investigation, metformin appears to improve the lipid profiles (total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol, except HDL-cholesterol) in diabetic rats.

In conclusion, this study indicates that metformin reduces DPP-IV and lipid levels (except HDL) and improves glucose tolerence in diabetic rats. Therefore, this study corroborates the earlier observation that metformin has DPP-IV inhibitory activity and it has anti-dyslipidimic effect, which is beneficial in type-2 diabetes mellitus with obesity. The present investigation thus encourages the development of new metformin analogues with better DPP-IV activity.

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References

1. DeFronzo RA, Bonadonna RC, Ferannini E. Pathogenesis of NIDDM: a balanced overview. Diabetes Care. 1992; 15: 318-368.
2. Drucker DJ. .Gut adaptation and the glucagon-like peptides. Gut.2002; 50:428-435.
3. Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Messeri G, Rotella C.M, et al. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. Diabetes Care. 2001; 24: 489-494.
4. Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide-1 *in vitro* and *invivo* by dipeptidyl peptidase-IV. Endocrinology.1995; 136:3585-3596.
5. Lambeir A-M, Durinx C, Scharpe´ S, De Meester I. Dipeptidyl - peptidase IV from bench to bedside: an update on structural properties, functions and clinical aspects of the enzyme DPP-IV. Crit Rev ClinLab Sci. 2003; 40: 209-294.
6. Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker K, Habener JF, Andersen DK, et al. The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7–37) in normal and diabetic subjects. Regul Pept. 1994; 51: 63 -74.
7. Stumvoll M, Nurjhan N, Periello G, Dailey G, Gerich J.E. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N. Engl. J. Med. 1995; 333: 550-554.
8. Cusi K, DeFronzo R.A. Metformin: a review of its metabolic effects. Diabetes Rev.1998; 6: 89-131.
9. Wu MS, Johnston P, Sheu WH. Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. Diabetes Care. 1990; 13(1):1-8.
10. Trinder P. Determination of blood glucose using an oxidase -peroxidase system with a non-carcinogenic chemogen. J Clin Pathol.1969; 22:158-161.
11. Durinx C, Neels H, Van der Auwera JC, Naelaerts K, Scharpe S, De Meester I, et al. Reference values for plasma dipeptidylpeptidase IV activity and their association with other laboratory parameters. Clin Chem Lab Med. 2001; 39:155-158.
12. Naito H.K. Coronary artery disease and disorders of lipid metabolism. Pesce L.A, Kamierczak, S.C. Clinical chemistry: Theory, analysis, correlation. 4th ed., St Louis USA, 2003: 603-614.
13. Brandt I, Joossens J, Chen X. Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by vildagliptin ((2S)-{[(3-hydroxyadamanta-1-yl) amino] acetyl}-pyrrolidine-2-carbonitrile). Biochem Pharmacol. 2005; 70:134 -143.
14. Ahren B. Gut peptides and type 2 diabetes mellitus treatment. Curr Diab Rep. 2003; 3:365-372.
15. Ahren B, Gomis R, Standl E, Mills D, Schweizer A. Twelve and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes Care. 2004; 27:2874 -2880.

16. Lindsay J.R, Duffy N.A, McKillop A.M, Ardill J, O'Harte F.P.M. Flatt P.R, Bell M, et al. Inhibition of dipeptidyl peptidase IV activity by oral metformin in type 2 diabetes. Diabet. Med. 2005; 22: 654 -657.
17. Yasuda N, Inoue T, Nagakura T, Yamazaki K, Kira K, Saeki T, Tanaka I, et al. Enhanced secretion of glucagon-like peptide-1 by biguanide compounds. Biochem. Biophys.Res.Commun. 2002; 298: 779-784.
18. Yong-Woon K, Jong-Yeon K, Yong-Hoon P, Kyu-Chang W, Kwang-Hae CH, Jung-Yoon H, Ki-Hak M, et al. Metformin restores leptin sensitivity in high-fat–fed obese rats with leptin resistance. Diabetes.2006; 55: 716-724.
19. Defronzo RA, Goodman AM. Efficacy of metformin in patient with non-insulin dependent diabetes mellitus. N Engl J Med. 1995; 333:541-549.
20. Chehade JM, Mooradian K. A rational approach to drug therapy of type-2 diabetes mellitus. Drugs. 2000; 60: 95-113.
21. Robinson AC, Burke J, Robinson S. The effects of metformin on glycemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. Diabetes Care. 1998; 21:701-705.

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