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Research Article

INHIBITION OF GLUCOSE LOWERING EFFECT OF SITAGLIPTIN ON CONCURRENT USE WITH AMLODIPINE ON ADRENALINE INDUCED HYPERGLYCEMIC CARDIOTOXIC RAT

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ABSTRACT

Objective- Sitagliptin is the first and only prescription medication in a new class of oral antihyperglycemic agents, which enhance the body's own ability to lower blood glucose when it is elevated. As many diabetic hypertensive patients taking amlodipine, the present study was undertaken to explore the effect of amlodipine on glucose lowering effect of Sitagliptin on adrenaline induced hyperglycemic cardiotoxic rats.Methods- Both acute and chronic effect of the drug combination was studied on adrenaline induced hyperglycemic cardiotoxic rats. In acute study, only glucose level was observed whereas in chronic study both glucose and lactate dehydrogenase (LDH) was estimated and heart was subjected to histopathological examination.Result- The glucose levels were increased significantly ($p<.05$) in adrenaline induced rats. Sitagliptin displayed significant reduction in rise in glucose level. The glucose level also significantly increased in rats where both Sitagliptin and amlodipine administered in both acute and chronic study. The rise in LDH level was also more in combination group in comparison to Sitagliptin alone.Conclusion- From the present study, it can be concluded that amlodipine inhibits glucose lowering effect of Sitagliptin in adrenaline induced hyperglycemic cardiotoxic rats.

Keywords: Sitagliptin, Amlodipine, adrenaline, glucose, cardiotoxicity.

INTRODUCTION

Diabetes is a group of disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. The symptoms of hyperglycemia include polyurea, polydypsea, weight, sometimes with polyphagia and blurred vision. Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of organs like eye, kidney, nerve, heart and blood vessels [1].According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. the three countries with the largest number of people with diabetes are India, China and the U.S.and India has largest number of diabetes patients [2].Hypertension is an extremely common co-morbid condition in diabetes, affecting nearly 20-60% of patients with diabetes, depending on obesity, ethnicity and age. In U.K. Prospective Diabetes Study (UKPDS) epidemiological study, each 10 mm Hg decrease in mean BP was associated with reductions in risk of 12% for any complication related to diabetes, 15% for death related to diabetes, 11% for myocardial infarction and 13% for microvascular complications [3].

Mortality is increased 7.2-fold when hypertension is present in patients with diabetes. In USA more than 75% of adults with diabetes have blood pressure (BP) levels >130/80 mm Hg are using antihypertensive medication [4]. The study revealed that calcium channel blockers particularly amlodipine and angiotensin converting enzyme inhibitors were the drugs of choice for hypertensives and diabetic hypertensives [5].

Sitagliptin is the first and only prescription medication in a new class of oral antihyperglycemic agents, which enhance the body's own ability to lower blood glucose when it is elevated. It is a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), which metabolizes the naturally occurring incretin hormones glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) resulting in enhanced glucose-dependent insulin secretion from the pancreas and decreased hepatic glucose production. Sitagliptin is not as likely to cause hypoglycemia as some other oral diabetes medications. Gliptins are largely weight neutral. No serious adverse events were noted during the clinical trials [6].

The calcium channel blockers can alter blood glucose level as calcium ions have a role in insulin secretion from pancreatic beta cells by the process of exocytosis [7]. So the aim of the present study is to find out the influence of amlodipine on the glucose lowering effect of Sitagliptin in normoglycemic and adrenaline induced hyperglycemic cardiotoxic rats.

MATERIAL AND METHODS

Drugs and Chemicals

Estimation of glucose was done by Glucometer (Onetouch Horizon, Johnson and Johnson ltd.)Lactate dehydrogenase was determined by standard methods using assay kit (Merck Diagnostic ltd. India).Sitagliptin (MSD Pharma Ltd) and Amlodipine (Amlodac, Zydus health care) were procured from market. Adrenaline was used to induce diabetes in rats was procured from market (Vasocon, Neon lab ltd).

Experimental Animals

Albino rats of either sex weighing 150-200 gm were used for the studies. Lightening was artificial in animal room.12-hour light and 12-hour dark sequence was maintained. Conventional laboratory diet was used for feeding with unlimited supply of drinking water by ad Libitum. This study was permitted by the Institutional Animal Ethical Committee with Reg. No. (1339 /ac / 10 / CPCSEA).

STUDY DESIGN

The whole study was completed in 2 phases. In the first phase the effect of drugs in acute doses and in second phase effect of drugs in chronic administration were established. In phase-1 only glucose level was estimated where as in phase-2 study glucose and LDH level was estimated. Histopathological study of cardiac tissue was done after chronic administration of drugs.

In each phase, the animals were divided into 4 groups (n=6).
Group-1: Normal control rats receive solvent (water) only.
Group-2: Cardiotoxic control group that Adrenaline (0.8 mg/kg) induced rats receive solvent.

Group-3: Cardiotoxic test group those Adrenaline induced rats treated with Sitagliptin (10 mg/kg).
Group-4: Cardiotoxic test group those Adrenaline (0.8 mg/kg) induced rats treated with Sitagliptin (10 mg/kg) and Amlodipine (5 mg/kg) combination.
Oral route of drug administration was followed using an intragastric tube. After 1 hour of drug treatment, the animals were administered with Adrenaline (0.8 mg/kg) by Intraperitoneal (IP) route [8]. The group-1 which was the control group was administered with solvent distilled water. After two hours blood was taken and glucose level was estimated by using a standard Glucometer.

Histopathological study

Cardiac tissues from all groups were subjected to histopathological studies. The whole heart from each animal was removed after sacrificing the animal under anesthesia and was collected in 10% formalin solution and immediately processed by paraffin technique. Section of 5µm thickness were cut and stained by hematoxylin and eosin (H & E) for histological examination [9]. The histopathological study was done in Dept. of Pathology, VSS medical college, Burla, Sambalpur, Odisha.

Statistical Analysis-The data obtained in the present study are expressed as Mean SEM and were analyzed by one way ANOVA followed by Dunnet's t-test. The values were considered statistically significant when $p<0.05$

RESULT

The Table -1 represents effect of single dose of sitagliptin and its combination with amlodipine on adrenaline induced hyperglycemic rats whereas Table-2 represents effect of chronic dose of sitagliptin and sitagliptin–amlodipine combination. In single dose treatment only glucose level was studied but in chronic treatment both glucose and LDH were estimated.

As shown in **Table-1**, adrenaline (0.8 mg/kg) significantly increases glucose level after 2hr of its administration. The glucose levels of animals (group-3) those are pretreated with single dose sitagliptin (10mg/kg) are much lower than glucose level of animals of group-2 (adrenaline alone). However there is no significant reduction in glucose level of animals (group-4) those are pretreated with both sitagliptin and amlodipine (5mg/kg).

The **Table-2** shows that the adrenaline causes both hyperglycemia as well as cardiotoxicity. The glucose and LDH level of animals those are pretreated with Sitagliptin alone (group-3) are very less in comparison to control group (group-2). Similarly the glucose and LDH level of animals those are pretreated with Sitagliptin and amlodipine (group-4) are not less in comparison to control group (group-2) i.e. the combination doesn't prevent hyperglycemia and cardiotoxicity caused by adrenaline.

Table 1: Acute effect of sitagliptin (10 mg/kg p.o.) alone and in combination with amlodipine (5 mg/kg p.o.) on blood glucose (mean ± SD) in adrenaline induced hyperglycemic rats.

Group	Treatment	Initial glucose(mg/dl)	Final glucose(mg/dl)
Group1	solvent	86.1±13.0	85.6±11.0
Group2	Adrenaline(0.5 mg/kg)	87.3±13.9	155.0±22.1*
Group 3	Sitagliptin+ Adrenaline	83.3±12.9	130.0±39.1*
Group 4	Sitagliptin+ Amlodipine+ adrenaline	85.6±14.7	201.0±53.8*

One way ANOVA followed by Dunnet's t-test. . n=6. *p<0.05Group II is compared with Group I, Group III, IV is compared with Group II and Group IV compared to Group III.

Table 2: Chronic effect of Sitagliptin (10 mg/kg p.o.) alone and in combination with amlodipine (5 mg/kg p.o.) on blood glucose (mean ± SD) in adrenaline induced hyperglycemic rats.

Group	Treatment	Glucose(mg/dl) (Mean±SD)	LDH(unit/lit) (Mean±SD)
Group1	Solvent	84.6±16.5	214.6± 37.4
Group2	Adrenaline(0.5 Mg/Kg)	162±15.5*	393±38.3*
Group 3	Sitagliptin(15day) + Adrenaline(15th Day)	112.3±15.7*	219±45.5*
Group 4	Sitagliptin+ Amlodipine15 Days+ Adrenaline(15th Day)	160.16±17.7*	296±54.9*

One way ANOVA followed by Dunnet's t-test. . n=6. *p<0.05
Group II is compared with Group I, Group III, IV is compared with Group II and Group IV compared to Group III.

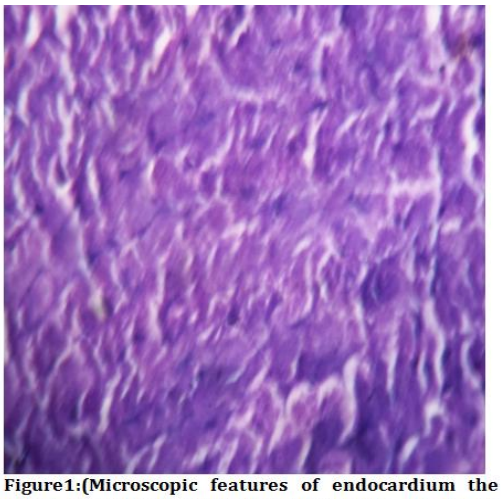

Figure1:(Microscopic features of endocardium the heart of animals treated with vehicle (Gr I). No necrosis found.

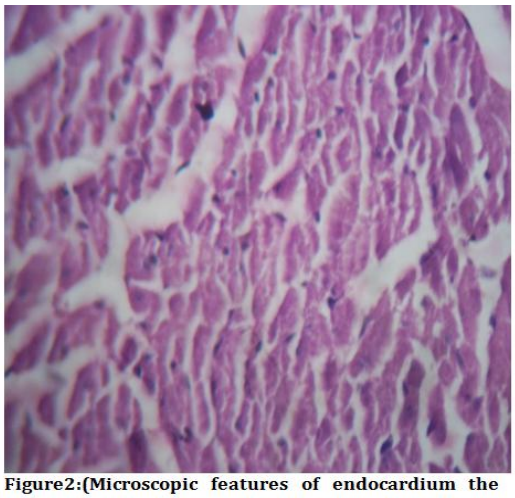

Figure2:(Microscopic features of endocardium the heart of animals treated with adrenaline (Gr II). Coagulative necrosis found.

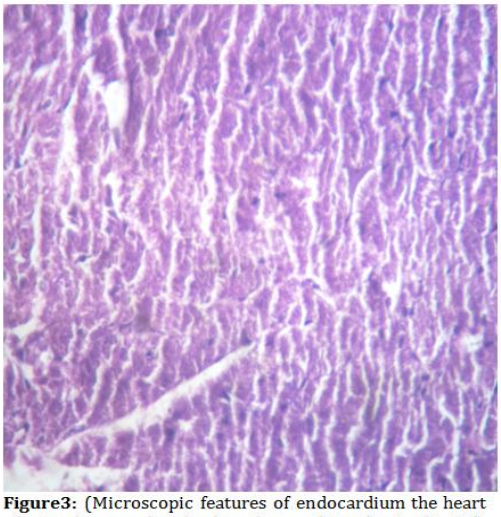

Figure3: (Microscopic features of endocardium the heart of animals treated with adrenaline and sitagliptin. (Gr III). No necrosis found.

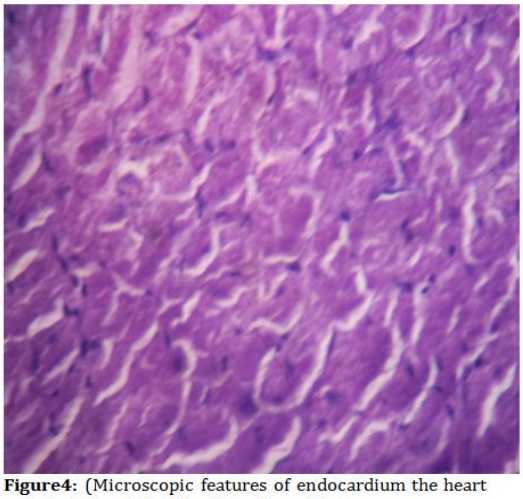

Figure4: (Microscopic features of endocardium the heart of animals treated with adrenaline with sitagliptin-amlodipine combination (Gr IV). No necrosis found.

Normal architecture of the endocardium was observed with no evidence of microscopic changes in the vehicle treated normal rats. Subepicardium and subendocardium region appears normal. In the heart of adrenaline treated rats gross pathological changes like black necrosis area of 1mm size in the ventricular wall found. The Subepicardium and subendocardium region show coagulative necrosis. But histological changes like necrosis were absent in the heart of rats of group-3 and group-4 those were pretreated with sitagliptin and sitagliptin-amlodipine combination respectively.

DISCUSSION

The present study was done to verify possible interaction between Sitagliptin and amlodipine when administered concurrently. The study was undertaken in both single dose treatment and repeated dose treatment in adrenaline induced hyperglycemic rats. Glucose level was considered as the parameter of the study. Sitagliptin was given in the dose of 10mg/kg [10] and Amlodipine in the dose of 5mg/kg [11].

Sitagliptin is the first prescription medication in a new class of oral antihyperglycemic agents, which enhance the body's own ability to lower blood glucose when it is elevated. It is a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), which metabolizes the naturally occurring incretin hormones glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) resulting in enhanced glucose-dependent insulin secretion from the pancreas and decreased hepatic glucose production. It doesn't cause hypoglycemia or any type of adverse effect. Also it has no in weight alteration [6]. Chronic sitagliptin treatment corrected the glycaemic dysmetabolism, hypertriglyceridaemia, inflammation and hypertension, reduced the severity of the histopathological lesions of pancreatic endocrine and exocrine tissues, together with a favourable redox status, which might be a further advantage in the management of diabetes and its proatherogenic comorbidities [10].
Since calcium is necessary for the secretion of insulin [11]. So calcium channel blockers are likely interact with the effect of antidiabetic drugs. Calcium channel blockers are widely used for the treatment of hypertension [12]. The calcium channel blocker like amlodipine reduced the blood glucose reduction by second generation sulfonylurea like gliclazide in alloxan induced diabetic animals when given simultaneously [13].
It has been shown CCBs have a strong intracellular "preventive" antioxidant effect. Chronic treatment of amlodipine (5 mg/kg, p.o.) in streptozotocin diabetic and spontaneous hypertensive rats causes prevention of streptozotocin induced hyperglycemia in both STZ-diabetic wistar and SH rats. The insulin level were decreased in non-diabetic treated wistar rats but were unaltered in non-diabetic SH and diabetic wistar and SH rats. Also there was significant reduction in cholesterol level in diabetic wistar and SH rats [11]. Chronic treatment of amlodipine in noninsulin dependent diabetic rats decrease in insulin release and reduction of glucose level occurs significantly which indicates amlodipine increases insulin sensitivity [14].
Calcium channel blockers appear to exert their cytoprotective effects through several mechanisms. These may involve blockade of L-(long-lasting)-type calcium channels, reduction of oxidative stress, antagonism at inflammatory mediator receptor sites and interaction at other intracellular sites. Studies relating to the liver are few but suggest that calcium channel blockers may have a role to play in limiting hepatocellular damage, especially those arising from exposure to a variety of toxic agents [15]. Amlodipine, a dihydropyridine derivative is used now a day for the treatment of hypertension. It has protective role against mitochondrial injury in ischemia and reperfusion injury which is proved in rats which may be due to antioxidant property [16]. Amlodipine was also proved to have high antioxidant effect among the calcium channel blockers [17].
As there is a possibility of use of both the drugs in case of patients suffering from diabetes with hypertension, it is designed to find out the safety of combination when used simultaneously. The study on adrenaline induced hyperglycemic cardiotoxic rat model is to validate same response in the actually used condition.
Epinephrine, via a beta adrenergic receptor mechanism, causes excessive plasma glucagon elevation and indicates that this hyperglucagonemia participates in the hyperglycemic property [18]. It also decreases insulin release by acting on alpha 2 receptors. Insulin increases mean BP in slow i.v. infusion or slow s.c. injection. In rapid i.v. injection (in animal) it causes marked increase in both systolic and diastolic BP [19]. It also causes myocardial infarction and increases serum AST, serum LDH level and myocardial MDA significantly ($p < .001$) in s.c. administration in the dose of 2mg/kg body weight [9].

The increased blood glucose concentration was not corrected in the group treated with both Sitagliptin and amlodipine as seen from the result. However the diabetic group that received only Sitagliptin, interestingly a significant reduction in the blood glucose level was noted. But the histopathological architecture of hearts of rats of both sitagliptin and sitagliptin-amlodipine treated group was normal.

CONCLUSION

Amlodipine being a calcium channel blocker, it blocks excess insulin release from the pancreas. But it doesn't inhibit normal insulin release when given to normoglycemic animals. The glucose as well as LDH levels were not corrected in combination group which may be due to inhibition of excess insulin release. Though amlodipine increases the insulin sensitivity in NIDDM rats, it is not effective in reducing the glucose level in adrenaline induced hyperglycemic rats when used in combination with sitagliptin than the effect of sitagliptin alone. The histopathological architecture of hearts of rats of both sitagliptin and sitagliptin-amlodipine treated group was

normal. It may be due to cytoprotective effect and protective role against mitochondrial injury in ischemia and reperfusion injury of amlodipine. The present study suggests concurrent use of amlodipine and sitagliptin combination should not be preferred to the patients those are suffering from diabetes co-existing hypertension or cardiovascular diseases.

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