

Adverse Effects of Combinatorial Therapy of Vildagliptin and Insulin on Cardiac Tissues in Diabetic Rats

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Abstract

Objectives: This study is aimed to evaluate the cardiovascular adverse effects of fixed dose combination of vildagliptin (10 mg/kg body weight) and insulin (0.27 I.U/kg body weight) on alloxan induced diabetic rats. **Methods and Analysis:** The cardiac tissues were weighed and homogenized using ice cold phosphate buffer, centrifuged and the supernatant used for further parameters. The different parameters were measured spectrophotometrically and statistical analysis of data was done by ANOVA followed by dunnet's post hoc test. The test samples consist of diabetic animal fed with vildagliptin and insulin as monotherapies and also as combinatorial therapy and the approach used for comparing them is by SPSS data analysis software version 19.0. **Findings:** The histopathological studies show that in combinatorial therapy of vildagliptin and insulin in alloxan an induced diabetic rat shows pathological lesions, inflammatory infiltration and eosinophilic cytoplasm. With regard to cardiac marker enzymes their levels were significantly elevated in combinatorial therapy showing cardiac damage. With regard to lipid profile the total cholesterol has been significantly reduced in combinatorial therapy showing it was not properly metabolized. These results were analysed by dunnet's post hoc test and the level of significance was set at $p < 0.05$. The drugs are producing severe adverse effects only at the post clinical trial stage. Our studies focus on the adverse effects produced by the drugs at the post clinical trial stage. Monitoring adverse drug reaction is still at a nascent stage owing to lack of awareness and healthcare professionals abstaining from reporting negative effects of pharmaceuticals. So our studies will be really helpful for developing new drugs which are devoid of adverse effects. **Applications and Improvement:** Our research will be a great resource for the pharmaceutical sector to develop new drugs which are devoid of all these adverse effects.

Keywords: Adverse Effects, Cardiac Tissues, Insulin, Incretins, Vildagliptin

1. Introduction

The World Health Organization (WHO) defines an adverse drug reactions as a response to drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of a disease, or for the modification of physiological function¹. ADR

represents one of the prime topics to be assessed in the modern times^{2,3}.

Diabetes Mellitus (DM) is a pandemic that affects more than 170 million people worldwide, associated with increased mortality and morbidity due to Coronary Artery Disease (CAD). Type 2 diabetes is nowadays an important problem in the society that needs to be managed

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in an efficient way. Many treatment options are available but they are often associated with many unpleasant side effects like hyperglycaemia, weight gain and loss of effectiveness of the drug in due course for example these may happen with the drugs like Sulphonylurea, Insulin, Thiazolidinedione etc. But a recent research about number of mechanisms in our body has led to the development of many new drugs which will act by increasing the intestinal hormones and thereby the problem of adverse effects will be greatly reduced⁴.

Many recent therapies for diabetes aim at the resistance produced by the body tissues to insulin and lack of functioning of beta cells for their activity. Incretins are a group of gastrointestinal hormones, predominantly Glucagon-Like Peptide-1 (GLP-1) chemokines, cytokines, and neuropeptides⁵. Vildagliptin is a very active and strong DPP4 inhibitor that will increase the activity of incretin hormones⁶. It enhances the glycemic control by stimulating the secretion of GLP-1 and GIP after the intake of food and also by delaying the degradation of incretins it increases the sensitivity of tissues to insulin and increases the activity of Betacells⁷. As a consequence of all its activities vildagliptin reduces to a great extent both the fasting glucose and the glucose level after taking food⁸. It also improves the ability of the body to regulate the glucose level so that establishing itself as a significant glucose lowering agent⁹. Vildagliptin alone can be given as monotherapy and also in combination with insulin.

But in combinatorial therapy, these oral hypoglycaemic agents are producing severe adverse effects causing damages to the various tissues in our body. In this article we have shown the damages to the cardiac tissues caused by this combinatorial therapy. Vildagliptin is contraindicated in patients with type 1 diabetes, diabetic ketoacidosis, severe liver impairment, during pregnancy and breastfeeding. But vildagliptin produces some Common side-effects such as: Central Nervous System-Dizziness, fatigue and feeling weak, chills, decreased blood sugar levels - very low blood sugar levels may lead to loss of consciousness, impairment of brain function, convulsions or death, nausea when vildagliptin has been taken in combination with metformin. The central nervous system which controls the entire motor unit, suffers degeneration that leads to many complications when treated with vildagliptin [oral anti-hyperglycemic agent of the new Dipeptidyl Peptidase-4 (DPP-4) inhibitor] treatment and produces severe neuromuscular degeneration. Till today this adverse effect of vildagliptin has not been proved. So

the main purpose of our research is to prove in vivo this cardiovascular adverse effect of vildagliptin.

2. Materials and Methods

2.1 Experimental Animals

Adult male Wistar Albino rats weighing 150-200 g of about 30 numbers were used. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature $25\pm 2^\circ\text{C}$ with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan lever, Kolkata, India) and water ad libitum. The animals were acclimatized to laboratory conditions for one month prior to experiment. All procedures described were reviewed and approved by the animal ethics committee, (IAEC No/03/006/2014).

2.2 Induction of Diabetes

By giving freshly prepared solution of alloxan to rats in fasting for 18 h in dosage of 150 mg/kg i.p they were induced for diabetes. 48 h after injection, the rats with blood glucose level above 250 mg/dl, were considered hyperglycemic and they were used for the study.

2.3 Experimental Design

The animals were divided into 5 groups consisting of 6 animals in each group.

Group I: Rats received normal saline (1ml/kgb.wt),

Group II: Rats administered with Alloxan (150 mg/kg/day) will be injected intraperitoneally as a single dose to induce diabetes.

Group III: Diabetic rats treated with vildagliptin (10 mg/kg b.wt) given orally as a single dose for 30 days.

Group IV: Diabetic rats treated with insulin (0.27 I.U/kg b.wt) administered i.m. as a single dose for 30 days.

Group V: Diabetic rats treated with same dose of insulin (0.27 I.U/kg b.wt) and Vildagliptin (10 mg/kg b.wt) for 30 days.

The heart rate was monitored in experimental group of rats using non-invasively designed acquisition setup. After monitoring, animals were sacrificed by injecting with sodium pentobarbitone and blood was collected in plain and heparinized tubes immediately after sacrifice for biochemical assays. Heart was removed and washed with saline. Blood samples centrifuged for 10 min at 2500 rpm and the serum separated stored at 4°C until further investigations.

2.4 Histological Examination

The heart tissue that were excised from the animals and were immersed in 10% formalin solution and after formal processing, they were embedded in paraffin wax and thin sections of 5µm thickness were cut down and stained using hematoxylin and eosin for microscopic examinations.

Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK)-MB enzyme levels in serum. The total cholesterol, phospholipid, triglyceride, free fatty acid content and lipoprotein levels was determined using standard Span diagnostic kits measuring in Shimadzu UV-Visible spectrophotometer.

2.5 Statistical Analysis

Results will be expressed as mean \pm S.E.M. Statistical significance is determined by one-way Analysis of Variance (ANOVA) and dunnet's post hoc test. P values less than 0.05 will be considered significant. For data processing SPSS data analysis software version 21.0 was used.

3. Results

3.1 Histology

Heart tissue of normal control rats showed normal cardiomyocytes with active myocardial fibres (Figure 1A). Sections from the animals treated with alloxan show interstitial edema and inflammatory infiltration between irregular wavy-directed cardiomyocytes (Figure 1B). Sections from the animals treated with alloxan and insulin show reduced inflammatory signs and recovery of normal cardiac fibres (Figure 1C). Sections from the animals treated with alloxan and vildagliptin show less inflammation in tissues compared with diabetic group (Figure 1D). Sections from the animals treated with alloxan and vildagliptin+insulin show pathological changes which include cardiac lesions, interstitial edemaeosynophylic cytoplasm and inflammatory infiltration between irregular wavy-directed cardiomyocytes (Figure 1E).

3.2 Cardiac Marker Enzymes

In the diabetic rats, levels of cardiac marker enzymes such as AST, ALT, LDH, CK-MB and ALP were elevated due to damage to the cardiac tissues in diabetic conditions

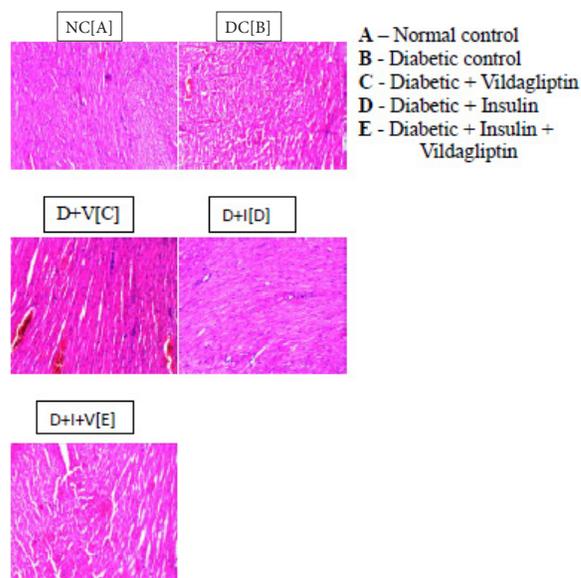


Figure 1. Showing effect of vildagliptin on cardiac tissues in alloxan-treated rats showing lesions in diabetic control and the ameliorative effect of insulin and vildagliptin as monotherapy and pathological lesions appear in combinatorial therapy.

compare to normal rats. In case of vildagliptin and insulin treated animals, the cardiac marker enzymes were to some extent reduced showing the little reduction in the damage to the cardiac tissues. But in combinatorial therapy of insulin and vildagliptin the cardiac marker enzymes elevation was significant in serum when compared to the monotherapy showing the damage to the cardiac tissues was severe in combinatorial therapy shown in Figure 2.

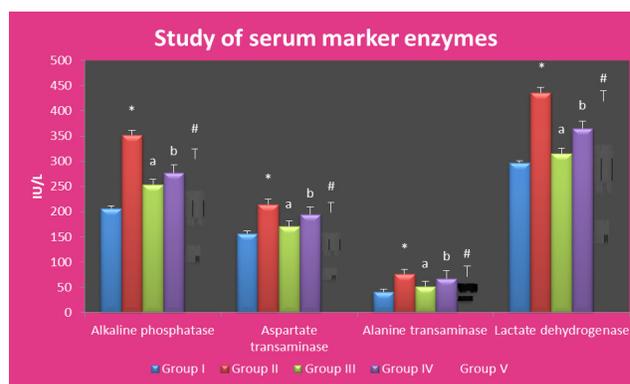


Figure 2. Depicting the level of serum marker enzymes on various groups.

* $P < 0.001$, statistically significant as compared with control rats: ^a $P < 0.001$; ^b $P < 0.01$, statistically significant as compared with diabetic rats and [#] $P < 0.001$, statistically significant as compared with normal.

3.3 Lipid Profile

In diabetic rats, the lipid levels have been increased significantly showing that the lipids were not properly metabolized. When treated with vildagliptin alone, the lipid profile which includes total cholesterol, free fatty acid, phospholipids and triglycerides were less significantly reduced compared to diabetic rats shown in Figure 3. When treated with insulin alone as a monotherapy, the metabolism of lipids has been moderately improved when compared to vildagliptin. But in combinatorial therapy the total cholesterol has been found to be significantly reduced but it does not show any effect on FFA and triglycerides when compared to the monotherapy.

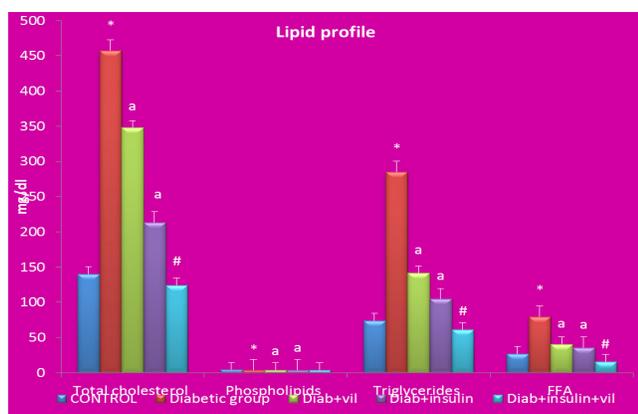


Figure 3. Depicting the level of different lipid levels on various groups.

* $P < 0.001$, statistically significant as compared with control rats; ^a $P < 0.001$; ^b $P < 0.01$, statistically significant as compared with diabetic rats and [#] $P < 0.001$, statistically significant as compared with normal.

4. Discussion

Evidence on the efficacy and safety of vildagliptin has been obtained from clinical studies, which were usually conducted in a restricted and highly regulated environment and may, thus, not necessarily reflect the everyday reality of diabetes management¹⁰.

Many people are using insulin with incretin based therapies. But mostly insulin produces hypoglycaemia and the incretin based therapies also have the potential for producing decrease in blood glucose level¹¹.

Mostly cardiovascular diseases are produced due to diabetes. If the patient has both Coronary Artery Disease (CAD) and diabetes their mortality rate will be greatly increased when compared to people without diabetes. UK

studies shows that good glucose control is associated with good cardiovascular health. But very intensive control of glucose will lead to low blood glucose levels.

Severe decrease in blood glucose level may increase the chances of cardiovascular adversities like myocardial ischemia and cardiac arrhythmia. The above said are only secondary to other adversities like constriction of blood vessels, increase in thickness and viscosity of blood¹².

The commonest causes of hypoglycaemia observed in hospital occur in diabetes and the use of insulin and other hypoglycaemic drugs, coronary atheroma, chronic renal failure, various tumours, different forms of liver disease, adrenal insufficiency, or hypopituitarism¹³. Evidence is accumulating that severe hypoglycaemia can provoke adverse cardiovascular outcomes such as myocardial ischemia or cardiac arrhythmia. Heart rate and systolic blood pressure are typically increased during a hypoglycaemia event¹⁴.

Cardiac markers are mostly used to measure cardiac function. Their levels are especially elevated in myocardial infarction. Mostly these are enzymes but some like troponins are not enzymes.

In the combinatorial therapy the cardiac marker enzymes have been elevated due to the cardiac tissue damage when compared to the monotherapies with insulin and vildagliptin.

With regard to the lipid profile in the combinatorial therapy when compared to the monotherapy the lipids are not effectively metabolized by the liver so the serum level of lipids are reduced and excess lipids are stored in the body without being metabolized. With regard to histopathology irregular cardiomyocytes with inflammatory infiltration have been observed in combinatorial therapy when compared to the monotherapies with insulin and vildagliptin.

The above parameters have shown that more damages have been observed in combinatorial therapy when compared to the monotherapies with insulin and vildagliptin.

5. Conclusion

The combinatorial therapy of insulin and vildagliptin have more intensified cardiovascular adverse effects when compared to the monotherapies with additional adversity on the metabolism of lipids and the adversity has also been evident in the cardiac tissues studied Histopathologically.

6. References

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