A study to evaluate the antihyperglycemic effect of ranolazine in rats with experimentally induced diabetes mellitus

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Abstract:

Background: Diabetes mellitus (DM) is a spectrum of common metabolic disorders characterized by hyperglycemia. Ranolazine is an active piperazine derivative, used as a first line agent in the treatment of chronic stable angina, either as a primary agent or as an adjunct to ongoing amlodipine, beta-blocker and nitrate therapy.[4] According to the CARISA (Combination Assessment of Ranolazine in Stable Angina) trial and its long-term open-label extension study, ranolazine significantly decreased HbA_{1c} values in patients with diabetes.[5,6] However, there are only few reports of experimental evaluation of the antihyperglycemic effect of ranolazine in rats. Hence, we have decided to undertake this study to evaluate the antihyperglycemic effect of ranolazine in rats with experimentally induced diabetes mellitus.

Methods: The animals selected for the study were experimentally naïve. The rats with following characteristics were selected:

Species: Rattus norvegicus ; Strain: Sprague-Dawley ; Sex: Male ; Body weight: 150-250 g. Dexamethasone was administered subcutaneously (s.c.) in the dose of 1 mg/kg/day to other groups, except normal control group for 5 days (days 6-10). The study drug ranolazine was administered per orally (p.o.) daily for a period of 10 days (days 1-10).

Results: Dexamethasone administration resulted in significant increase in fasting blood glucose levels. Ranolazine administration lead to a significant reduction in fasting blood glucose levels compared to dexamethasone group ($p<0.05$).
Administration of ranolazine significantly lowered the insulin resistance, when compared to dexamethasone control group ($p<0.05$).

Conclusion: Administration of dexamethasone (1 mg/kg/day for 5 days s.c.) to rats resulted in significant hyperglycemia and development of insulin resistance. The antihyperglycemic effect of ranolazine was observed in this study.

Keywords: Diabetes mellitus, hyperglycemic, ranolazine, experimental studies, anti-hypergylcemic effect

INTRODUCTION

Diabetes mellitus (DM) is a spectrum of common metabolic disorders characterized by hyperglycemia. Factors contributing to its pathogenesis include insufficient insulin secretion, reduced responsiveness to insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. Chronic complications from prolonged hyperglycemia include retinopathy, neuropathy, nephropathy and cardiovascular disease.[1]

Diabetes mellitus is one of the leading causes of morbidity and mortality worldwide. An estimated 422 million people worldwide, corresponding to 8.5% of the world's adult population, lived with diabetes in 2014.[2] This is projected to increase to 642 million by the year 2040. With an estimated 69.2 million people living with diabetes, India has the world's second largest diabetes population.[3]

Currently, the management of diabetes mellitus includes, insulin and oral anti-diabetic drugs (OAD) like sulfonylureas, biguanides, thiazolidinediones, glinides, alpha-glucosidase inhibitors, dipeptidyl peptidase 4 (DPP-4) inhibitors, and sodium glucose co-transporter 2

(SGLT-2) inhibitors. Oral anti-diabetic drugs are usually the first line of drug treatment for type-2 diabetes mellitus. A combination of two or more of the above drugs is usually required, in order to achieve optimal blood glucose levels. However, each of these classes of drugs has some limitations like hypoglycemia, weight gain, peripheral edema, etc.

Ranolazine is an active piperazine derivative, used as a first line agent in the treatment of chronic stable angina, either as a primary agent or as an adjunct to ongoing amlodipine, beta-blocker and nitrate therapy.[4]

According to the CARISA (Combination Assessment of Ranolazine in Stable Angina) trial and its long-term open-label extension study, ranolazine significantly decreased HbA_{1c} values in patients with diabetes mellitus.[5,6] However, there are only few reports of experimental evaluation of the antihyperglycemic effect of ranolazine in rats.

Hence, we have decided to undertake this study to evaluate the antihyperglycemic effect of ranolazine in rats with experimentally induced diabetes mellitus.

2. AIM AND OBJECTIVES

2.1 AIM

To evaluate the antihyperglycemic effect of ranolazine in rats with experimentally induced diabetes mellitus.

2.2 OBJECTIVES

To study the antihyperglycemic effect of ranolazine in rats with experimentally dexamethasone induced diabetes mellitus.

3. MATERIAL AND METHODS

3.1 ETHICAL CONSIDERATIONS:

The study commenced after IAEC (Institutional Animal Ethics Committee) approval was granted and was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.64

3.2 ANIMALS AND THEIR MAINTENANCE:

3.2.1 Animals used:

The animals selected for the study were experimentally naïve. The rats with following characteristics were selected:

Species: Rattus norvegicus

Strain: Sprague-Dawley

Sex: Male

Body weight: 150-250 g.

The rats used in the present study were procured from Animal house, Dr. D.Y. Patil Medical College, Hospital & Research Centre, Pimpri, Pune-411018.

3.2.2 Animal feed:

Food: Animals were fed with commercially available 'Nutrimix Std-1020' manufactured by Baramati Agro Ltd., acquired from Nutrivet Life Sciences, Pune. The nutrition provided by the pellet feed was as follows:

Energy: 3620 kcal/kg

Crude protein: 22.15%

Crude fibre: 62.48%

Ash: 5.11%

Sand silica: 1.15%

Pellets were kept in the space provided for feed on the roof of the cage.

Water: Drinking tap water supplied by Pimpri Chinchwad Municipal Corporation was provided to the rats through the feeding tubes with stainless steel nozzle, one per rat cage.

Both food and water were replenished once daily in the morning, and were available to the rats ad libitum.

3.2.3 Animal housing:

Rats were housed in groups of four in standard big polypropylene cages measuring 40 X 27.5 X 13.5 cm, which had a wire mesh top with provision for drinking water and space for pellets. Corncob was used as bedding material in each cage.

The rats were housed under standard conditions of temperature (25 ± 5°C), relative humidity (55 ± 10%), and 12/12 hour light/dark cycle. Apart from daily replenishment of food and water, rats were left undisturbed.

3.3 STUDY DRUGS AND DOSES:

1. **Ranolazine**
   Dose: 90 mg/kg body weight[65]
   Source: Sun Pharmaceutical Industries Ltd. (Avior), Mumbai.

2. **Dexamethasone sodium phosphate**
   Dose: 1 mg/kg body weight[66]
   Source: Laborate Pharmaceuticals India Ltd.

3.4 STUDY PROTOCOL:

3.4.1 Dexamethasone induced diabetes mellitus

Dexamethasone was administered subcutaneously (s.c.) in the dose of 1 mg/kg/day to other groups, except normal control group for 5 days (days 6-10).

The study drug ranolazine was administered per orally (p.o.) daily for a period of 10 days (days 1-10).

3.4.2 Grouping:

Rats selected at random were divided into following groups. (n=8 each group)

Group	Type	Dose & route
I	Normal control	Distilled water 1 ml p.o.
II	Dexamethasone control	Dexamethasone 1 mg/kg s.c.
III	Dexamethasone + Ranolazine	Dexamethasone 1 mg/kg s.c. and Ranolazine 90 mg/kg p.o.

3.4.3 Measurement of blood glucose levels:

3.4.3.1 Schedule:

On day 11 of study, after overnight fasting, blood samples were collected from the tail vein of rats for estimation of blood glucose levels using a glucometer (Accu-chek, Roche Products Pvt. Ltd.).

3.4.3.2 Rat tail vein blood withdrawal:

The animal was placed in a rat holder. The tail vein was made prominent by applying xylol. A needle (Gauge 23) was used to prick the tail. The non-dominant hand was placed to make the tail immobile using index finger and thumb. The glucometer with test strip inserted was kept ready. Tail vein was pricked and the drop of blood was collected on the test field of the strip. Pressure with cotton gauze was applied to stop the bleeding. Care was taken not to waste any extra blood than needed. The rat was placed back in the respective cage for recovery.

3.4.4 Calculation of HOMA-IR:

Insulin resistance was calculated by Homeostatic Model Assessment method, using HOMA2 calculator version 2.2.3.[67]

3.5 STATISTICAL ANALYSIS:

The data was compiled and analyzed using the statistical package, Primer of biostatistics version 5.0. Results were expressed as Mean ± S.E.M. Statistical significance between means was analyzed using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. *P value* <0.05 was considered to be statistically significant.

4. OBSERVATIONS AND RESULTS

As per the protocol, study was carried out in three different groups of rats. Results are expressed as Mean ± S.E.M.

4.1 Effect on blood glucose levels:

Table 5.1 shows effect of study drugs on fasting blood glucose levels (mg/dl) on day 11.

Table 4.1: Fasting blood glucose levels on Day 11

Group	Treatment	BSL-F (mg/dl)
I.	Normal control	75.88 ± 2.28
II.	Dexamethasone control	139 ± 3.12$^{@}$
III.	Dexamethasone + Ranolazine	100.5 ± 2.95*
@ p<0.05, in comparison to group I; * p<0.05, in comparison to group II		

Dexamethasone administration resulted in significant increase in fasting blood glucose levels. Ranolazine administration lead to a significant reduction in fasting blood glucose levels compared to dexamethasone group (p<0.05).

4.2 Effect on serum C-peptide levels:

Table 5.2 shows effect of study drugs on fasting serum C-peptide levels (ng/ml) on day 11.

Table 4.2: Serum C-peptide levels on day 11

Group	Treatment	Serum C-peptide levels (ng/ml)
I.	Normal control	0.29 ± 0.04
II.	Dexamethasone control	0.83 ± 0.03
III.	Dexamethasone + Ranolazine	0.79 ± 0.01

Serum C-peptide levels in normal rats varies from 0.24 to 0.72 ng/ml.[68] Dexamethasone administration resulted in increase in serum C-peptide levels. Serum C-peptide levels between ranolazine group and dexamethasone control group were comparable.

4.3 Effect on HOMA-IR:

Table 5.3 shows effect of study drugs on HOMA-IR on day 11.

Table 4.3: HOMA-IR on day 11

Group	Treatment	HOMA-IR
I.	Normal control	0.63 ± 0.14[69]
II.	Dexamethasone control	0.70 ± 0.02
III.	Dexamethasone + Ranolazine	$0.60 \pm 0.01^{*}$

Dexamethasone administration resulted in development of mild insulin resistance, as suggested by mild elevation in HOMA-IR. Administration of ranolazine significantly lowered the insulin resistance, when compared to dexamethasone control group ($p<0.05$).

5. DISCUSSION

Ranolazine is an FDA approved drug used in the treatment of chronic stable angina. CARISA, MERLIN-TIMI 36, and TERISA trials have shown its favourable cardiovascular safety profile in type 2 DM patients.[5,47,70] Post-hoc analyses of these trials showed that ranolazine might have antihyperglycemic effects, as evidenced by a reduction in HbA_{1c}.[6] Since, there are only a few reports on experimental evaluation of the antihyperglycemic effects of ranolazine, we decided to undertake this study.

In the present study, dexamethasone was used to induce diabetes in rats. Subcutaneous administration of dexamethasone is a well-established model of insulin resistance and hyperglycemia. Dexamethasone induces whole body insulin resistance by targeting mainly skeletal muscle, liver and adipose tissue thereby decreasing peripheral glucose utilization and

increasing hepatic glucose output.[59] It has been used in the dose range of 1 to 10 mg/kg for a period of 5-10 days.[55-58,66] We used dexamethasone in the dose of 1 mg/kg/d for a period of 5 days, which resulted in mild insulin resistance and hyperglycemia. (Table 5.1 and 5.3)

Administration of ranolazine (90 mg/kg) resulted in significant decrease in blood glucose levels and HOMA-IR, suggesting its antihyperglycemic effect. Previously, Ning Y et al[50] have reported that administration of ranolazine (20mg/kg/day for 8 weeks) to streptozotocin-induced diabetic mice resulted in significant reduction in fasting plasma glucose levels as compared to control group of mice (187 ± 19 mg/dl vs. 273 ± 23 mg/dl). In another study conducted by Bashir S et al,[71] ranolazine was administered to streptozotocin-induced diabetic albino wistar rats in the dose of 45 mg/kg daily for a period of 28 days. The study showed that the blood glucose levels in ranolazine-treated group (206.3 ± 12.74 mg/dl) were significantly lower than the control group (437.8 ± 34.3 mg/dl) at day 28.
The antihyperglycemic effect of ranolazine was seen.

Apart from animal studies, two phase III clinical trials were conducted to evaluate the efficacy of ranolazine in type 2 DM patients. Eckel RH et al[49] studied the effect of ranolazine monotherapy on glycemic control in patients of type 2 DM. At week 24, ranolazine significantly lowered HbA_{1c} (mean difference -0.56%) and fasting blood glucose levels (mean difference -8mg/dl) compared to placebo. Also, fasting serum C-peptide levels were significantly lower in the ranolazine group as compared to placebo at week 24 (baseline difference -0.20 ng/ml). In the present study, HbA_{1c} was not evaluated. But, the effects of ranolazine on fasting blood glucose and serum C-peptide levels were similar to those observed in this clinical trial. Although we did not study the mechanism of action of the antihyperglycemic effect of ranolazine, a few reports of its probable mechanism of action are available in literature.

Dhalla AK et al[51] have studied the mechanism of action for the antihyperglycemic effect of ranolazine in rats. It was observed that ranolazine reduces the postprandial and basal glucagon levels by inhibiting the $Na_v1.3$ isoform of sodium channel present on pancreatic α-cells. The findings were similar to those after administration of a more selective Na^+ channel blocker (GS-458967). There was an association between reduction of hyperglycemia and glucagon levels. This suggests that the sodium channel blockade might have prevented the secretion of glucagon from the pancreatic α-cells. Ning Y et al[50] conducted a study, which showed that

administration of ranolazine to streptozotocin treated mice increased β-cell survival and enhanced insulin secretion in a glucose dependent manner.

Hence, further studies are needed to explore the antihyperglycemic effect of ranolazine with measurement of important parameters like HbA_{1C}, serum glucagon levels, and histopathology of pancreas.

Due to its antianginal and antihyperglycemic action, ranolazine can be considered a novel agent with dual action. It could play a vital role in the treatment of patients suffering from type 2 DM and chronic stable angina together.

6. CONCLUSION

From the present study, we can conclude the following:

- Administration of dexamethasone (1 mg/kg/day for 5 days s.c.) to rats resulted in significant hyperglycemia and development of insulin resistance.
- The antihyperglycemic effect of ranolazine was observed in this study.

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