Influence of Rimonabant on oral hypoglycemic activity of Glibenclamide and Glipizide on albino & diabetic rat

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ABSTRACT

The present study was planned to study the influence of Rimonabant pretreatment (1 week) on the anti-diabetic activity of oral anti-diabetic agents in albino & Streptozotocin induced diabetic rats. Rimonabant (0.36mg/kg) & Sulfonylureas like Glibenclamide (180µg/kg) and Glipizide (270µg/kg) were administered and the time to onset of hypoglycemia, duration of the hypoglycemia & peak hypoglycemia were determined. Then Rimonabant (0.36mg/kg) was administered for 1week and on the next day 1 hour after Rimonabant treatment, the hypoglycemic effect of Sulfonylureas were evaluated. The results showed that co-administration of Rimonabant with Glipizide and Glibenclamide had positive influence on the peak hypoglycemic effect and a significant enhancement in the duration of hypoglycemic action in both normal and diabetic rats. This increased hypoglycemic activity of Glipizide and Glibenclamide after Rimonabant treatment may be due to improved insulin sensitivity and other insulin-resistant state in diabetes.

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Introduction

Obesity and diabetes mellitus (DM) are major causes of morbidity and mortality in the US and other countries[1, 2]. Evidences indicates that obesity and weight gain are associated with an increased risk of diabetes[3, 4] and that intentional weight loss reduces the risk that overweight people will develop diabetes[5]. Each year, an estimated 300000 US adults die of causes related to obesity[6], and diabetes is the sixth leading cause of death. Correspondingly, both obesity and diabetes generate immense health care costs[7, 9].

DM leads to severely debilitating or fatal complications, such as heart disease, blindness, kidney disease and amputations. In type I DM, the pancreas stops producing insulin due to autoimmune response or possibly viral attack on pancreas. It develops as a result of the synergistic effects of genetic, environmental, and immunologic factors that ultimately destroy the pancreatic β cells. Type II DM or non insulin-dependent DM or adult-onset diabetes is characterized by three pathophysiological abnormalities: impaired insulin secretion; peripheral insulin resistance, and excessive hepatic glucose production.

Obesity is characterized by excess body fat to such an extent that health may be negatively affected. It is commonly defined as a body mass index of 30 kg/m^2 or higher. This distinguishes it from being overweight as defined by a BMI of between 25-29.9[10]. Many studies show an association between excessive body weight and various diseases, particularly cardiovascular diseases, DM Type II, sleep, apnea, certain types of cancer and osteoarthritis. As a result, obesity has been found to reduce life expectancy. With rates of adult and childhood obesity increasing, authorities view it as a serious public health problem. Attempts to address it include population-wide measures to improve dietary choices and increase physical exercise[11]. Obesity, particularly visceral or central (hip-waist ratio), is very common in type II DM and occurs at middle age[2].

As general observations about 90-95 % of people suffering with DM are Type II; about 80 percent are overweight[12]. Adipocytes secrete a number of biologic products (leptin, TNF-α, free fatty acids, resistin, and adiponectin) that modulate insulin secretion, insulin action and body weight and may contribute to the insulin resistance. Ultimately β cell failure may ensure[13].

In clinical situation, obesity and type-II DM may occur in a single patient necessating the treatment for the both simultaneously[14]. To keep tight glycemic control, oral antidiabetic agents like sulfonylureas are required[15, 16]. On the other hand to reduce the obesity associated with elevated blood lipid levels; suitable anti-obesity drug has to be used. However, when two or more drugs are used concurrently in a single patient there is every possibility of occurrence of drug-drug interaction between some of the administrated drugs, which may range from mild to very severe.

Materials and Methods

Experimental animals- Albino rats of either sex weighing between 150-250 gm were selected for the study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC No.576/2002/bc/IAEC/CPCSEA) and animal were maintained under standard condition in animal house approved by Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs- Rimonabant (Torrent Pharmaceuticals Ltd., Ahmedabad, India); Glipizide and Glibenclamide (Dishman Pharma Ltd., Ahmedabad, India); Streptozotocin (Sd- Fine-chemicals pvt. Ltd., Mumbai, India) Glucose Estimation Kit (Auto Span Liquid Gold, Span Diagnostics Ltd., Surat, India) and Autoanalyzer (Erba Mannheim chem-5 plus V2) were used in the present study. All the drugs used in this study were administered by suspending separately in 2% w/v gum acacia suspension in distilled water.

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Induction of diabetes- The 2 groups of 6 albino rats were administered with 65mg/kg of streptozotocin dissolved in citrate buffer (0.1 M, pH 4.5) i.p. After 24 hours, blood samples were collected for determination of fasting glucose concentration. The rats with blood glucose level above 350 mg/dL were considered to be diabetic and were used in the experiment.

Sample Collection- The blood samples were collected by tail vein method [17, 18] at a time intervals (0, 0.5, 1, 2, 4, 8, 12, 18 and 24 hours) and centrifuged for 10 minutes at 5000 rpm, decanting supernatant fluid into the clean, dry test tube. Blood glucose level was estimated with the help of GOD/POD method using Erba Mannheim chem -5 plus V_2 autoanalyser [19].

Evaluation of hypoglycemic action

(i) **Influence of Rimonabant on blood glucose levels in normal albino rats-** Suspension of Rimonabant (0.36mg/kg) in 2%w/v of gum acacia was administered orally at the morning to all the rats (n=6) for one week. On the 7th day, 6 hours after administration of Rimonabant, the rats were fasted for 18 hours. On the 8th day, the blood samples were collected after the administration of Rimonabant at different time intervals up to 24 hours.

(ii) **Effect of Rimonabant pre-treatment on the hypoglycemic activity of Glipizide and Glibenclamide in normal albino rats**- In the first part, the healthy rats of either sex (n=6) were divided into 2 groups. One received suspension of Glipizide (270μg/kg) in 2%w/v of gum acacia and other received Glibenclamide suspension (180μg/kg) through oral route and blood samples were collected at different time intervals (0, 0.5, 1, 2, 4, 8, 12, 18 and 24 hours).

In the next part of this experiment, all the healthy rats were treated with Rimonabant (0.36mg/kg) orally for one week. On the 7th day, 6 hours after administration of Rimonabant, the rats were fasted for 18 hours. On the 8th day blood samples were collected for determining fasting blood glucose levels and Rimonabant (0.36mg/kg) was administered orally to all the animals. After 60 minutes, Glipizide (270μg/kg) or Glibenclamide (180μg/kg) was administered to animals. Blood samples were collected thereafter at different time intervals (0, 0.5, 1, 2, 4, 8, 12, 18 and 24 hours).

(iii) **Effect of Rimonabant pre-treatment on the antidiabetic activity of Glipizide and Glibenclamide in diabetic rats**- In the first part, the diabetic rats in a group (n=6) of 2 received suspension of Glipizide (270μg/kg) & Glibenclamide (180μg/kg) through oral route and blood samples were collected by tail vein.

In the next part, all the diabetic rats were treated with Rimonabant (0.36mg/kg, p.o. once a day) for one week. On the 7th day, 6 hours after administration of Rimonabant, the rats were fasted for 18hours. On the 8th day, Rimonabant (0.36mg/kg) was administered orally to all the animals. After 60 minutes, Glipizide (270μg/kg) & Glibenclamide (180μg/kg) were administered to animals.

Blood samples were collected thereafter at 0.0, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 18.0 and 24.0 hours and analyzed for glucose levels by using GOD/POD method which was expressed as mg/100ml of blood. Then the hypoglycemic activity of Glibenclamide and Glipizide at time't' was calculated and the % of blood glucose reduction at various time intervals were calculated before and after Rimonabant treatment.

$$\% \text{ Blood glucose reduction at time 't'} = \frac{A-B}{A} \times 100$$

Where,

A = Initial blood glucose level before drug administration.

B = Blood glucose levels at time't' after the drug administration.

Results

Effect of Rimonabant per se treatment on blood glucose levels in healthy albino rats- Treatment of Rimonabant (0.36mg/kg) had positive influence on the blood glucose levels (32.37 ±1.40% reduction) in normal albino rats. The onset of action was observed between 1 to 2 hours and duration of action was 12 hours. This indicates that Rimonabant has a hypoglycemic effect in healthy rats. The results are compiled in Table 1 and graphically depicted in Fig. 1.

Fig.1- Effect of Rimonabant (0.36 mg/Kg) per se treatment on blood glucose levels in normal albino rats
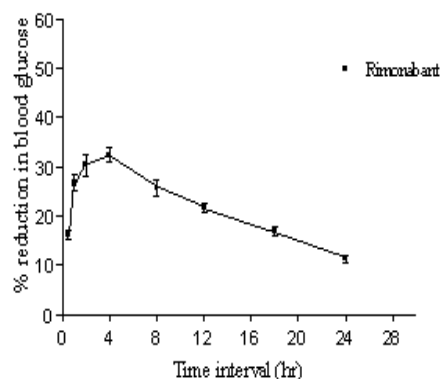
Influence of Rimonabant pre-treatment on Glipizide and Glibenclamide induced hypoglycemia in healthy albino rats- Rimonabant pre-treatment (0.36 mg/kg p.o.) once a day for 7 consecutive days with Glipizide and Glibenclamide had not significantly altered the onset of hypoglycemia (1 hour before treatment and 1 hour after treatment). Glipizide significantly affected peak hypoglycemia (39.32 ± 1.08% before treatment and 46.46 ± 0.93% after treatment), however duration of hypoglycemia was observed 12 hrs before treatment and 18 hrs after treatment.

Glibenclamide enhanced the peak effect (41.44 ±0.54% reductions before treatment to 51.81 ±0.74 % reductions after treatment) and duration of hypoglycemia was also enhanced from 12 hrs to 24 hrs. The results of these findings are recorded in Table 1 and graphically depicted in Fig. 2, 3.

Fig. 2- Influence of Rimonabant (0.36mg/kg) on hypoglycemia induced by Glipizide in healthy albino rats.
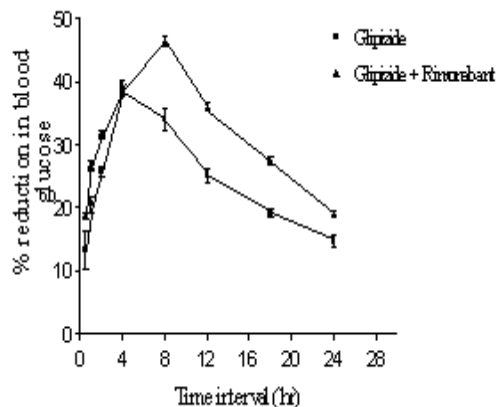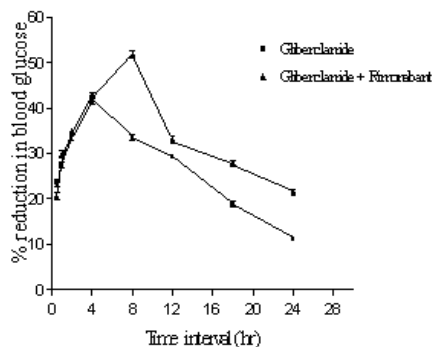
Fig. 3- Influence of Rimonabant (0.36mg/kg) on hypoglycemia induced by Glibenclamide in normal albino rats

Influence of Rimonabant pre-treatment on the Glipizide and Glibenclamide induced hypoglycemia on diabetic albino rats

In diabetic rats, pre-treatment of Rimonabant (0.36 mg/kg, p.o.) once a day for 7 consecutive days, with Glipizide and Glibenclamide significantly decreased onset of hypoglycemic action (from 1 hour before treatment to 0.5 hour after treatment). Glipizide increased peak hypoglycemia ($35.19 \pm 0.44\%$ reduction before treatment to $44.21 \pm 0.29\%$ after treatment) and duration of hypoglycemia was enhanced from 12 to 24 hours. Also Glibenclamide increased peak hypoglycemia ($41.65 \pm 0.38\%$ before treatment and $50.14 \pm 0.41\%$ after treatment) and duration of hypoglycemia was increased from 12 to 24 hours. The results of these findings are compiled in Table 2 and graphically depicted in Fig. 4, 5.

Fig. 4- Influence of Rimonabant (0.36mg/kg) on hypoglycemia induced by Glipizide in diabetic rats
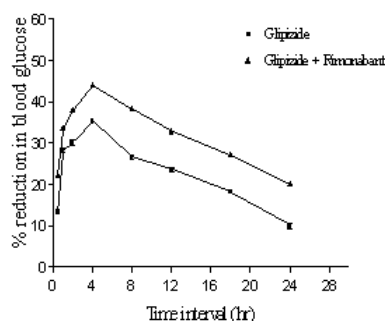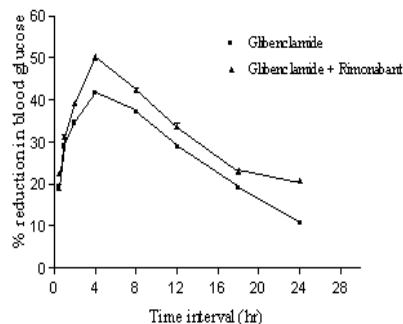

Fig. 5- Influence of Rimonabant (0.36mg/kg) on hypoglycemia induced by Glibenclamide in diabetic rats

Discussion

Obesity and DM are two common chronic conditions which frequently coexist and can significantly affect individual health care needs. Rimonabant, the first selective cannabinoid type1 (CB1) receptor antagonist is approved drug for treatment of obesity[20]. It can help people with diabetes by reducing their risk of dyslipidemia, stroke, hypertension and other micro-vascular complications. It is evident that CB1 receptors are expressed in pancreatic islets and skeletal muscles[21]. Since, CB1 receptor inhibitor, Rimonabant could improve the blood circulation and glucose uptake in skeletal muscles, increased basal oxygen consumption and modulate glucose homeostasis, thus favoring peripheral insulin action. Patients on Rimonabant therapy reduce both micro vascular and macro vascular complications in diabetes and appear to improve insulin sensitivity and glucose metabolism. There is evidence that pharmacological treatment with Rimonabant affords special benefits, not only in patients after myocardial infarction and in congestive heart failure but also in persons with hypertension accompanied with the cardio metabolic syndrome and type II DM. Concurrent use of Rimonabant and anti-diabetic agents usually appears to produce hypoglycemia, but uneventful. Increased hypoglycemic and anti diabetic activity of Glipizide and Glibenclamide after Rimonabant treatment may be due to improved insulin sensitivity and other insulin-resistant state in diabetes as these are reported with Rimonabant[20]. The results showed that pre-treatment of Rimonabant in diabetic rats has shortened the onset of antidiabetic activity and enhanced the duration of action from 12 to 24 hours of Glipizide as well as Glibenclamide. Also it causes significant increase in peak hypoglycemic effect of Glipizide and Glibenclamide i.e. from 35.19 % to 44.21 % and from 41.65% to 50.14% respectively. From the results obtained, it can be indicated that repeated treatment of Rimonabant exerts a definite additive or synergistic pharmacodynamic effect on hypoglycemic/ antidiabetic action of Glipizide and Glibenclamide, not only in normal state but also in diabetes conditions. It was observed from result that Rimonabant appears primarily to give additive or synergistic pharmacodynamic effect with enhanced insulin sensitivity by utilization of glucose in skeletal muscles, increase in micro and macro vascular circulation to pancreas and decreased insulin resistance.

Conclusion

Rimonabant pre-treatment increases the hypoglycemic and anti-diabetic activity of Glipizide and Glibenclamide. The additive or synergistic pharmacodynamic drug-interaction between Rimonabant and antidiabetic drugs like Glipizide and Glibenclamide must be considered during their long term treatment in diabetic patients and proper dose adjustment to avoid severe hypoglycemia.

References

1. Must A, Spadano J, Coakley E H, Field A E, Colditz G & Dietz W H, The disease burden associated with overweight and obesity. JAMA, 282 (1999) 1523-1529.
2. Pi-Sunyer F X, Health implications of obesity, Am J Clin Nutr, 53 (1991) 1595-1603.
3. Ford E S, Williamson D F & Liu S, Weight change and diabetes incidence: findings from a national cohort of US adults, Am J Epidemiol, 146 (1997) 214-222.
4. Resnick H E, Valsania P, Halter J B & Lin X, Relation of weight gain and weight loss on subsequent diabetes risk in

overweight adults, J Epidemiol Comm. Health, 54 (2000) 596-602.

5. Will J C, Williamson D F, Ford E S, Calle E E & Thun M J, Intentional weight loss and 13-year diabetes incidence in overweight adults, Am J Public Health, 92 (2002) 1245-1248.

6. Allison D B, Fontaine K R, Manson J E, Stevens J & VanItallie T B, Annual deaths attributable to obesity in the United States, JAMA, 282 (1999) 1530-1538.

7. Colditz G, Economic costs of obesity and inactivity, Med Sci Sports Exerc, 31 (1999) 663-667.

8. Ricordeau P H, Weill A, Vallier N, Bourrel R, Schwartz D, Guilhot J, Fender P & Allemand H, The prevalence and cost of diabetes in metropolitan France: what trends between 1998 and 2000, Diab. Metab, 29 (2003) 497-504.

9. Mokdad A H, Bowman B A, Ford E S, Vinicor F, Marks J S & Koplan J P, The continuing epidemics of obesity and diabetes in the United States, JAMA, 286 (2001) 1195-1200.

10. Janghorbani M & Amini M, Comparison of body mass index with abdominal obesity indicators and waist-to-stature ratio for prediction of type II diabetes: The Isfahan diabetes prevention study, Obesity Research & Clinical Practice, 4 (2010) 25-32.

11. Ehtesham N Z, Molecular link between diabetes and obesity: The resist in story, Current Science, 80 (2001) 10-11.

12. Pearson W S, Ten-year comparison of estimates of overweight and obesity, diagnosed diabetes and use of office-based physician services for treatment of diabetes in the United States, Preventive Medicine, 45 (2007) 353-357.

13. Nyholm B, Porsen N, Juhl C B, Gravholt C H, Butler P C, Weeke J, Veldhuis J D, Pincus S & Schmitz O, Assessment of insulin secretion in relatives of patients with type 2 (non-insulin dependent) diabetes mellitus: Evidence of early β cell dysfunction, Metabolism, 49 (2000) 896-905.

14. Johnson R A, Abdominal obesity and ethnic differences in diabetes awareness, treatment and glycemic control, Clinical Cornerstone, 5(2) (2003) 64.

15. Butterfield W J H, Whichelow M J, Effect of diet, sulphonylureas and phenformin on peripheral glucose uptake in diabetes and obesity, The Lancet, 292 (7572) (1968) 785-788.

16. Lean M E J, Management of Obesity and Overweight, Medicine, Elsevier Ltd. 34(12) (2006) 515-520.

17. Satyanarayana S, Prasad K & Nitin M, Pharmacodynamic drug interaction of selegiline and tolbutamide in rats, Indian drugs, 40(6) (2003) 366-368.

18. Eswar Kumar K, Ramesh A & Satyanarayana S, Pharmacodynamic and Pharmacokinetic Drug Interaction of Gliclazide and Lacidipine in Animal Models, Ind J. Pharm. Educ. Res, 42 (3) (2008) 277-281.

19. Trinder P, Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor, Ann. Clin. Biochem, 6 (1969) 24-7.

20. Gadde K M, Allison D B, Cannabinoid-1 Receptor Antagonist, Rimonabant, for Management of Obesity and Related Risks, J Ame. heart society, 114 (2006) 974-984.

21. André J S, Nicolas P, Inhibitors of cannabinoid receptors and glucose metabolism, Current Opinion in Clin. Nutr.& Metabol Care, 11(4) (2008) 505-511.

Table 1- Influence of Rimonabant and its pre-treatment on Glipizide and Glibenclamide induced hypoglycemia in healthy albino rats

Treatment Time(Hrs)	0.5	1	2	4	8	12	18	24
Rimonabant	16.18±0.94	26.66±1.61	30.36±2.14	32.37±1.40	25.75±1.63	21.66±0.96	16.99±0.83	11.13±0.82
Glipizide	13.23±3.11	20.50±1.19	25.72±0.70	39.32±1.08	34.03±1.73	25.18±1.10	19.20±0.56	14.73±0.94
Glipizide + Rimonabant	18.73±0.55	26.78±0.74**	31.67±0.62***	38.19±0.51	46.46±0.93***	36.02±0.66***	27.46±0.70***	19.11±0.51***
Glibenclamide	23.60±0.80	27.46± 0.56	33.61± 0.61	41.44±0.54	33.46± 0.55	29.27± 0.45	18.83± 0.56	11.17± 0.42
Glibenclamide + Rimonabant	20.52±0.72*	29.78±0.73*	34.84± 0.46*	42.83±0.53	51.81±0.74***	33.14±0.78**	27.77±0.77**	21.55±0.64**

Paired "t" test; n=6, * Significant at $p<0.05$; ** highly significant at $p<0.01$; *** Very highly significant at $p<0.001$

Table 2- Influence of Rimonabant pre-treatment on the Glipizide and Glibenclamide induced hypoglycemia on diabetic albino rats

Treatment Time(Hrs)	0.5	1	2	4	8	12	18	24
Glipizide	13.40± 0.31	28.28± 0.58	30.17± 0.76	35.19± 0.44	26.74± 0.54	23.62± 0.62	18.19± 0.42	9.93± 0.63
Glipizide + Rimonabant	22.16±0.37***	33.72±0.40***	38.07±0.49***	44.21±0.29***	38.38±0.41***	32.89±0.37***	27.39±0.52**	20.16±0.39***
Glibenclamide	19.23± 0.79	28.99± 0.42	34.51± 0.42	41.65± 0.38	37.15± 0.44	28.88± 0.28	19.15± 0.48	10.67± 0.34
Glibenclamide + Rimonabant	22.39± 0.49**	31.19± 0.67*	39.25±0.28***	50.14±0.41***	42.28±0.56***	33.75±0.61***	23.04± 0.47**	20.87±0.45***

Paired "t" test; n=6, * Significant at $p<0.05$; ** highly significant at $p<0.01$; *** Very highly significant