

Research Article

Pharmacodynamic Drug Interaction of Repaglinide with Atorvastatin in Wistar Albino Rats

Maulika Patel^{1*}, Mihir Patel², Nimmy Chacko¹ and Shastry CS¹

¹Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Manglore, Karnataka, India.

²Department of Pharmacology, STE'S Smt.Kashibai Navale College of Pharmacy, Kondhwa (Bk), Pune. India.

ABSTRACT

The present study is aimed to explore the pharmacodynamic interaction of Repaglinide with Atorvastatin in rats. Diabetes was induced by 35 mg/kg streptozotocin in wistar albino rats (male 200-250 g) fed with high fat diet, they were divided into six groups, each consisting of six rats. Normal control group (1) is treated with 1%w/v carboxy methyl cellulose (CMC). Group 2 served as diabetic control. To the diabetic 3rd, 4th group Repaglinide(MTD) and atorvastatin (MTD) were administered orally respectively for 8 weeks. The combination of Repaglinide(TD)+ atorvastatin(MTD) and Repaglinide(MTD)+ atorvastatin(MTD) were administered to the 5th and 6th group of diabetic rats for 8 weeks. On the last day blood samples were collected, from tail vein and cardiac puncture serum was isolated and subjected to Blood glucose, triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), Very low density lipoprotein (VLDL), high density lipoprotein (HDL) and Atherogenic index were estimated as well as liver enzyme marker such as AST, ALT and ALP were estimated. Body weight was also calculated. Results revealed that, Repaglinide significantly reduced the serum glucose level in diabetic rats. Atorvastatin produced mild hypoglycemia. Repaglinide (MTD) + Atorvastatin treated group shows hypoglycemic effect as well as marginal increase in the body weight. Atorvastatin significantly reduced the serum TG, TC, LDL and VLDL and increased HDL level. Repaglinide also altered the lipid profile of diabetic rats. Whereas the combination of Repaglinide(TD) + atorvastatin(MTD) and Repaglinide(MTD) + atorvastatin(MTD) significantly reduced the lipid profile when compared to atorvastatin alone. Atherogenic index of diabetic control shows higher risk of cardiovascular disorder compare to atorvastatin treated animal. The antidiabetic drug repaglinide enhanced the hypolipidemic activity of atorvastatin. Similarly atorvastatin enhanced the hypoglycemic activity of Repaglinide due to pharmacodynamic interactions. Repaglinide (MTD) + Atorvastatin (MTD) treated group showed highly significant increase in ALT, AST and ALP and by histopathological study it showed lipid changes in hepatocytes which indicates early liver damage. Marginal escalation in ALT, AST and ALP level in repaglinide (TD) + atorvastatin (MTD) treated groups, whereas histopathological studies showed mild hydropic changes but no severe hepatocytes damage in this group. Hence caution should be exercised during co-prescribing these drugs in humans. Clinicians should take precautions and monitor their patients for signs of adverse effects or decreased efficacy and titrate dosages accordingly.

Keywords: Repaglinide, Atorvastatin, Diabetes, Hyperlipidemia, Pharmacodynamic.

INTRODUCTION

Diabetes is a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complication from vascular disease. Among diabetics, approximately 95% of patients have type 2 diabetes mellitus(DM), whereas about 5% of patients have type 1 diabetes mellitus(DM). Patients with DM are at risk

for microvascular complication like retinopathy, nephropathy and neuropathy and macrovascular complications like myocardial infarction that increase morbidity and mortality.¹ Diabetes patients may also be affected with many other diseases like hypertension, cardiovascular disease, Peptic ulcer and fungal infections etc. which require prolong treatment.² There are reports that dyslipidemia is likely more prone to develop with

patients suffering from diabetes.³ The characteristic features of diabetic dyslipidemia are high plasma triglyceride concentration, low HDL-C and increased concentration of LDL-C.⁴ Repaglinide a carbamoylmethyl benzoic acid derivative which was developed to specifically control meal-related glucose fluctuations in patients with type 2 DM. Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase used in patients with diabetes and hypercholesterolemia.⁵ Repaglinide is metabolized by cytochrome CYP3A4 in liver and Atorvastatin also metabolized by the same in liver. Atorvastatin is potent inhibitor of cytochrome CYP3A4.⁶ Literature review revealed that co-administration of repaglinide and atorvastatin has greater pharmacokinetic interaction and atorvastatin has decreased repaglinide metabolism thereby increasing plasma repaglinide concentration.⁷ These two drugs when co-prescribed which are either inducers or inhibitors of CYP3A4 may lead to hepatotoxicity.

In this paper we have evaluated pharmacodynamic and CYP3A4 interaction of atorvastatin and repaglinide. Our present study aims to identify metabolic changes of lipid and liver function test when these two drugs are prescribed together.

MATERIALS AND METHODS

Animals

Male Wistar albino rat weighing between 200 to 250 gm were selected for the experiment. Animals were procured from Animal house, NGSMIPS, Manglore, India. They were housed in polypropylene cages with not more than six animals per cage and maintained under standard conditions (12 h light/12 h dark cycle; 25 ± 3°C; 35–60% humidity). Animals were allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. The study was approved by Institutional Animal Ethical Committee (IAEC).

Streptozotocin induced diabetes

All experimental animals were allocated into high fat diet (HFD) regimens consisting pork fat and normal animal food pellet (NAFP) for initial period of 15 days. Compositions of HFD are as follows:

a) NAFP(powdered) - 150 gms/kg

b) Animal (Pork) fat- 75 gms/kg

After 15 days of dietary manipulation, all rats from each high fat dietary group was injected intraperitoneally (i.p.) with freshly prepared solution of streptozotocin (STZ) (35mg/kg) in 0.1 M citrate buffer (pH 4.5). The rats with the non-fasting plasma glucose level of ≥ 300 mg/dl were considered diabetic and selected for further pharmacological studies. Blood glucose level was estimated by using blood glucose monitoring system (glucometer). A drop of blood was collected from rat tail vein. Accuracy of glucometer was estimated by comparing its result with quantitative determination of blood glucose by GOD-POD method.⁸

Doses

Dose of Repaglinide and Atorvastatin for animal was selected on the basis of previous literature review. Thus, repaglinide therapeutic dose (0.2 mg/kg p.o) and maximum therapeutic dose (0.4 mg/kg p.o) while atorvastatin maximum therapeutic dose (10 mg/kg p.o) was given once a day.

Treatment

Animals were divided into four groups (n=6). Each group was subjected for eight week treatment with respective drug.

Group I served as Normal control is treated with 1%w/v carboxy methyl cellulose (CMC).

Group II served as HFD-Diabetic control

Group III served as HFD-Diabetic rats treated with repaglinide (MTD)

Group IV served as HFD-Diabetic rats treated with atorvastatin (MTD)

Group V served as HFD-Diabetic rats treated with repaglinide (TD)+ atorvastatin (MTD)

Group VI served as HFD-Diabetic rats treated with repaglinide (MTD)+ atorvastatin (MTD)

On the last day blood samples were collected, from tail vein and cardiac puncture serum was isolated for assessment of different enzyme activities. The rats were then sacrificed by cervical dislocation the liver was carefully dissected, cleaned of extraneous tissue. Liver tissue samples were fixed in 10 % formalin for pathological examination.

Estimation of liver marker enzymes and Lipid Profile

AST, ALT and ALP were estimated for hepatic damage.^{9, 10} Serum total cholesterol, Serum triglycerides, HDL-C, LDL-C and VLDL-C were estimated for their metabolic influence by this drug-drug interaction.^{11, 12, 13}

Estimation of Body weight, Blood Glucose level and Atherogenic Index

After eight week treatment body weight and blood glucose level was estimated and analysed to compare with baseline value. Atherogenic Index was calculated by using formula: $(\text{Total Cholesterol-HDL-C})/\text{HDL-C}$

Histopathology of liver

Portions of liver were preserved in 10% neutral buffered formalin for 24 hr. Specimens were dehydrated and embedded in paraffin, sectioned at 6 μ m and stained with hematoxylin and eosin (H&E) for histopathological examination.¹⁴

Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Dunnet test against control. The results were considered statistically significant for $p < 0.05$.

RESULTS

Effect on liver biomarker enzymes

Significant ($p < 0.001$) increase in AST, ALT and ALP was observed in repaglinide treated group when compared with control. While rise in these levels was also significant in group V and group VI when compared against repaglinide treated group. (Table 1)

Effect on Lipid Profile

Increased level of total cholesterol, serum triglycerides, LDL-C and VLDL-C in repaglinide treated group was found significant ($p < 0.001$) when compared with control and significant ($p < 0.001$) decrease in total cholesterol, serum triglycerides, LDL-C and VLDL-C level in group II, group III, group IV, group V and group VI was also observed when compared with diabetic control. Level of HDL-C was significantly increased in group II, group III, group IV, group V and group VI ($p < 0.001$) when compared with diabetic control. (Table 2)

Effect on Body weight, Blood Glucose level and Atherogenic Index

The body weight was slightly increased in the normal control rats, whereas in the diabetic rats there was a significant reduction in body weight is due to poor glycemic control and impaired carbohydrate metabolism. Group III, group IV, group V and group VI treatment significantly prevented this reduction in the body weight of animals in these groups. (Figure 1) Group III and group V showed significant decrease in blood glucose level when compared with group II. (Figure 2) Group III, group IV, group V and group VI showed significant decrease in atherogenic index when compared with group II. (Figure 3)

Histogram of liver

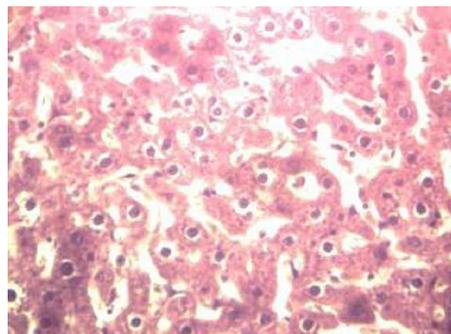


Fig. 4: Section show liver tissue of Control groups

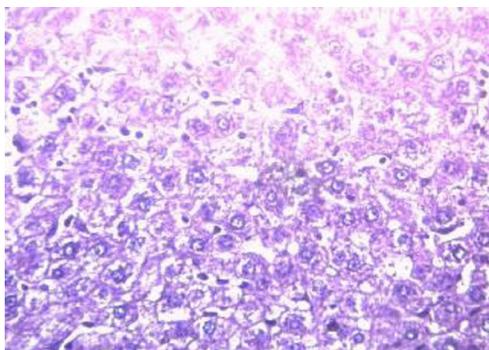


Fig. 5: Section show liver tissue of Repaglinide (MTD) treated group

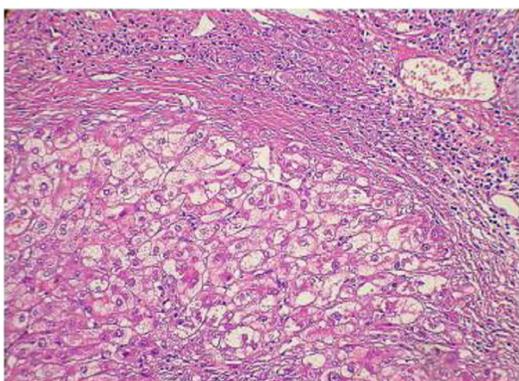


Fig. 6: Section show liver tissue Repa (TD) + ATV treated group

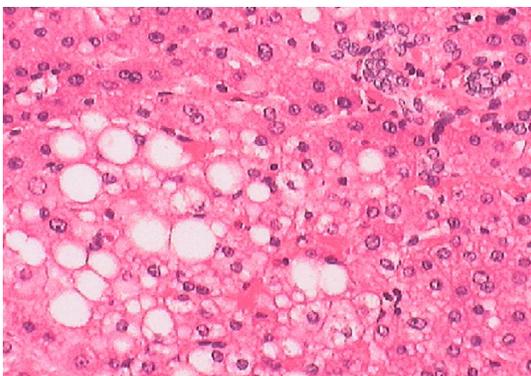


Fig. 7: Section show liver tissue of Repa (MTD) + ATV treated group

DISCUSSION

The present study was designed to evaluate antidiabetic potential and change in lipid profile of repaglinide in combination with atorvastatin. Diabetic rats showed increase in blood glucose levels than control. In Repaglinide treated (MTD) group highly reduction in blood glucose level was observed. On the other hand the treatment with atorvastatin slightly

decreased the Blood glucose level, but not that much as repaglinide does, when compared with Repaglinide treated group. Whereas treatment with combination of repaglinide(MTD)+ atorvastatin decreased the blood glucose concentration lower than Repaglinide treatment (hypoglycemia) which is statically significant.

The result revealed that there was significant decrease in blood glucose level in group treated with repaglinide (TD) + atorvastatin when compared to group treated with repaglinide alone. Hypoglycemia was observed in groups treated with repaglinide (MTD) + atorvastatin, and when compared with Repaglinide treated group. The body weight was slightly increased in the normal control rats, whereas in the diabetic rats there was a significant reduction in body weight is due to poor glycaemic control and impaired carbohydrate metabolism. Repaglinide(MTD), atorvastatin(MTD) and in combination of Repaglinide(TD) + atorvastatin(MTD) and Repaglinide(MTD) + atorvastatin(MTD) treatment significantly prevented this reduction in the body weight of animals in these groups. Marginal escalation in body weight was observed in repaglinide treated group (i.e., 0.67%). Although there is a marginal reduction in the body weight of animals in atorvastatin(MTD) and in combination of Repaglinide(TD) + atorvastatin(MTD) and Repaglinide(MTD) + atorvastatin treatment groups compared to initial body weights it fell short of statistical significant. However the reduction in the body weight was significant when compared to the final weight of normal control rats.

Streptozotocin treatment not only increases blood glucose levels but also increases the levels of triglycerides, total cholesterol and LDL-C, VLDL-C in diabetic rats. Diabetic rats showed increase in the serum levels of triglycerides, total cholesterol, LDL-C and VLDL-C and decreased HDL-C level when compared to control. Atorvastatin decreased the lipid profile near to normal control, which is statistically significant. Repaglinide also

slightly altered the lipid profile. On the other hand the treatment with combination of repaglinide (TD) + atorvastatin and repaglinide (MTD)+ atorvastatin (MTD) on diabetic rats decreased serum triglycerides, total cholesterol and LDL-C, VLDL-C levels and increased HDL-C levels than atorvastatin treatment, which is statistically significant. Atherogenic index is higher in diabetic animal which indicates there is a higher risk for cardiovascular disease atherosclerosis. Repaglinide (TD) + atorvastatin (MTD) and repaglinide (MTD) + atorvastatin (MTD) treated group shows significant decrease in atherogenic index which shows better hyperlipidemic activity in this group and there is no risk of atherosclerosis when compared to atorvastatin treated group. The result revealed that, there was highly significant elevation of AST level in repaglinide (MTD) + Atorvastatin treated groups when compared with Repaglinide alone treated group. Whereas, slight elevation was seen with Repaglinide (TD) + atorvastatin treated group when compared with repaglinide treated group alone. There was very highly significant elevation of ALT level in group treated with repaglinide(MTD) + atorvastatin (MTD) when compared with repaglinide treated group alone. No significant change in ALT level was observed in repaglinide (TD) + atorvastatin treated group when compared with repaglinide treated group alone. There was highly significant elevation of ALP level in repaglinide (MTD) + atorvastatin treated group, when compared with repaglinide treated group alone. No significant change in ALP level was observed in repaglinide (TD) + atorvastatin treated groups. Histopathological study of liver section revealed that, in control group animals, normal lobular architecture and normal

histology were maintained; in the group treated with repaglinide alone at MTD, no such significant damage or change in hepatocytes were observed. In repaglinide (TD) + atorvastatin treated group, mild hydropic change was observed; but there was no portal inflammation or hepatocytes necrosis was observed. In repaglinide (MTD) + atorvastatin treated group, fatty change was observed. It is indication of early damage of hepatocytes. Large and clear vacuoles in the cytoplasm of hepatocytes were observed.

CONCLUSION

The antidiabetic drug repaglinide enhanced the hypolipidemic activity of atorvastatin. Similarly atorvastatin enhanced the hypoglycemic activity of Repaglinide due to pharmacodynamic interactions. Atorvastatin inhibits microsomal enzyme CYP3A4, so, there will be not much metabolism of Repaglinide by CYP3A4, and it leads gradually escalation of free Repaglinide concentration in blood. High concentration of Repaglinide in blood causes, excessive insulin secretion which leads hypoglycemia. Hence cautions should be exercised during co-prescribing these drugs in humans. If concomitant use of drugs that potentially change CYP3A4 activity is necessary in patients treated with Repaglinide, clinicians should take precautions and monitor their patients for signs of adverse effects or decreased efficacy and titrate dosages accordingly.

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TABLE 1: Effect on AST, ALT and ALP enzymes

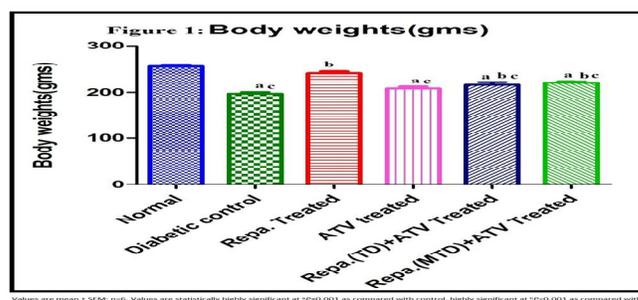
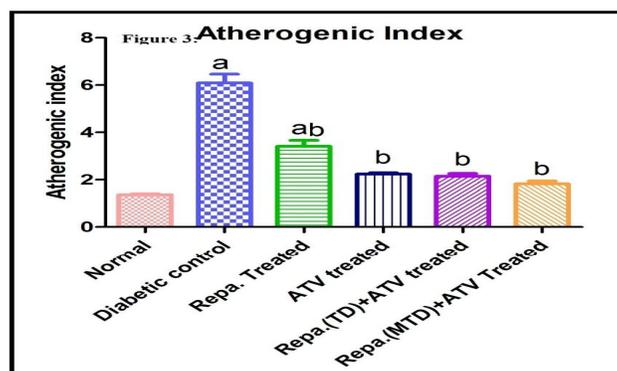
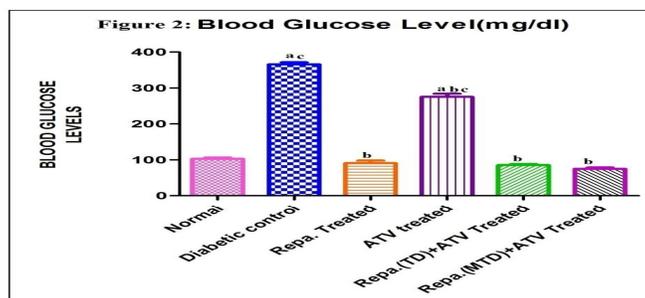
GROUPS	AST	ALT	ALP
Control	51.65±2.12	27.22±1.49	509.5±10.78
REPA. Treated	69.17±1.37 ^{***}	56.56±3.10 ^{***}	578.6±25.56
REPA.(TD)+ATV (MTD)	90.76±3.52 ^{####}	69.94±2.41 ^{***}	640.2±19.28 ^{***}
REPA(MTD)+ATV(MTD)	122.30±3.78 ^{####}	98.85±2.78 ^{####}	1050±23.65 ^{***}

Values are mean ± SEM; n=6. Values are statistically highly significant at ^{***}P<0.001 as compared with control and highly significant at ^{####}P<0.001 as compared with REPA. Treated group

TABLE 2 : Effect on Total cholesterol, Serum triglycerides, HDL-C, LDL-C and VLDL-C level

Groups	TC	TGs	HDL-C	LDL-C	VLDL-C
Normal	114.6±3.28 ^{bc}	75.59±1.73 ^{bc}	48.62±2.11 ^b	50.87±1.02 ^{bc}	15.12±0.35 ^{bc}
Diabetic Control	179.2±2.89 ^{bc}	157.8±2.57 ^{bc}	26.31±1.36 ^{bc}	121.4±3.55 ^{bc}	31.56±0.51 ^{bc}
Repa. treated	150.4±3.16 ^{bc}	121.9±2.19 ^{bc}	34.59±1.67 ^b	91.58±3.94 ^{bc}	24.22±0.35 ^{bc}
ATV treated	130.8±1.53 ^{ab}	93.05±4.05 ^{ab}	40.53±0.67 ^b	71.86±1.94 ^{ab}	18.48±0.88 ^{ab}
Repa.(TD)+ATV treated	123.7±1.70 ^b	90.14±4.09 ^{ab}	39.47±1.18 ^b	64.96±1.91 ^{ab}	18.03±0.81 ^{ab}
Repa.(MTD)+ATV treated	118.9±2.12 ^{abc}	90.10±2.61 ^{ab}	42.46±1.71 ^b	58.44±2.67 ^{bc}	18.02±0.52 ^{ab}

Values are mean ± SEM; n=6. Values are statistically highly significant at ^{***}P<0.001 as compared with control, highly significant at ^bP<0.001 as compared with Diabetic control and highly significant at ^cP<0.001 as compared with Repa. Treated group.



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