

Lipid Lowering Effect of Teneagliptin in Comparison to Simvastatin in Diet Induced Hyperlipidemic Rats

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Gliptins are drugs that inhibit the enzyme dipeptidyl peptidase-4 and are one of the current treatment options for type 2 diabetes mellitus. The study aimed to compare the lipid lowering effects of teneagliptin with simvastatin in diet induced hyperlipidemic rats. 22 Sprague Dawley male rats were administered high fat diet over a period of 4 weeks to induce hyperlipidemia, after which, 10 rats each in groups 1 and 2 were orally gavaged with simvastatin (standard) and teneagliptin (treatment) respectively for 2 weeks. 2 rats in group 3 were vehicle controls. Blood samples were collected at baseline, at the end of 4 weeks and 6 weeks to determine the levels of total cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol. Histopathology of liver and aorta of 2 control animals and 3 animals each from the groups 1 and 2 was done at the end of the study. A paired t test showed a statistically significant reduction in mean total cholesterol ($p < 0.001$), triglycerides ($p < 0.001$) and low density lipoprotein cholesterol ($p = 0.003$), but no significant change in mean high density lipoprotein cholesterol ($p = 0.796$) in teneagliptin group at the end of 6 weeks compared to the pre-treatment values. There was no statistically significant difference between the simvastatin and teneagliptin groups in all the four lipid parameters studied. Compared to baseline, there was a rise in body weight after induction and a reduction post-treatment in both groups which did not achieve statistical significance. Histopathology of liver in both groups 2 and 3 demonstrated reversal of the congestion and mild fatty changes which were apparent in the control group while sections of aorta failed to reveal any significant changes even in the control group. The lipid lowering property of teneagliptin demonstrated in this study will be therapeutically beneficial in type 2 diabetic patients with dyslipidemia.

Keywords: Dipeptidyl Peptidase4 Inhibitors; Dyslipidemia; Hypolipidemic Activity; Teneagliptin.

Hyperlipidemia is a metabolic disorder that involves abnormally increased levels of lipids and lipoproteins in the blood. Most dyslipidemias

(80%) are related to diet and lifestyle, although familial disorders (20%) are important as well. The American Heart Association estimates a prevalence

of 11.9% which is around 28.5 million adults = 20 years of age having serum total cholesterol levels = 240 mg/dl, which is considered a high level necessitating treatment.¹ A study conducted by Indian Council of Medical Research shows a high prevalence in India with approximately 79% of the 16607 study subjects manifesting abnormalities in at least one of the lipid parameters.²

Hyperlipidemia is one of the major risk factors that cause arteriosclerosis, cerebral stroke, coronary heart disease, myocardial infarction and renal failure. Statins are widely used to lower cholesterol levels because of their inhibitory effects on the 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis. The most important adverse effects of statins are increased concentrations of liver enzymes, myopathy, rhabdomyolysis and increased risk of diabetes.^{3,4} In addition, reports of an increase in the potential risk of cognitive impairment with statins have been documented.⁵

Teneligliptin is a dipeptidylpeptidase-4 inhibitor that inhibits the enzyme dipeptidylpeptidase-4 (DPP-4) and is a potent treatment option for type 2 diabetes (T2DM) either as monotherapy or in combination with other hypoglycemic agents. Gliptins are used for the treatment of T2DM when patients fail to show adequate glycemic control after dietary changes and exercise or with a combination of lifestyle modification and metformin /sulfonylurea treatment.^{6,7} They have relatively lesser adverse effects, weight neutral and do not cause any significant hypoglycemia.^{7,8} Dyslipidemia is an important risk factor for cardiovascular complications in type 2 diabetic patients. It is unclear whether gliptins favorably improve lipid profile in type 2 diabetic patients as there are contradicting evidences from clinical studies.^{9,10}

The DPP-4 inhibitors are also currently being evaluated for their effects on obesity and other metabolic traits.^{11,12} In addition, their role in chronic liver diseases including non-alcoholic steatohepatitis is also under investigation.^{13,14}

In animal studies, a beneficial effect on blood lipids has been detected in type 2 diabetic animal models like Zucker fatty rats. studies indicate a potential lipid lowering effect of teneligliptin in T2DM, the efficacy of the drug as

an independent anti-hyperlipidemic agent is not yet determined. Thus the aim of this study was to evaluate the lipid lowering effect of teneligliptin in high fat diet rat model as there is paucity of studies assessing the hypolipidemic action of gliptins in diet induced hyperlipidemia model. So, in this study, we compared the effect of gliptins (teneligliptin) to that of statins (simvastatin) on hyperlipidemia induced in rats using high fat diet.

MATERIALS AND METHODS

After the approval of the Institutional Animal Ethics Committee (Approval number: 288/2015/IAEC), the study was done in the department of Pharmacology at a Medical college and research institute in Coimbatore. 22 healthy adult Sprague Dawley male rats of weight 180 g to 250g and age more than 2 months were chosen for the study. They were housed in polypropylene cages at normal ambient temperature and 12-hour light dark cycle. The animals were administered high cholesterol diet over a period of 4 weeks to induce hyperlipidemia after which, with 2 rats as controls, 10 rats were randomly allotted to each of the groups 1 and 2 following which they were administered the drug/vehicle during the last two weeks (5th, 6th) along with normal standard chow diet.

Standard group (1): 10 rats given simvastatin (10mg/kg/day)

Treatment group (2): 10 rats given teneligliptin (20 mg/kg/day)

Control group (3): 2 rats given vehicle (sterile water)

The composition of high-lipid diet consisted of 1% (w/w) cholesterol, 10% (w/w) fat lard, 0.2% propylthiouracil, 5% yolk and 1% sodium tauroglycocholate.¹⁵ The rats were allowed the high fat diet and water ad lib. The drugs simvastatin at a dose of 10 mg/kg and teneligliptin at a dose of 20 mg/kg were dissolved in sterile water and the control animals were given the vehicle (sterile water) all of which were administered by oral gavage.

Blood samples (1.5 ml) were collected from tail vein of each rat using tail snip method under anesthesia. Blood sample collection was done at baseline, at the end of 4th week and 6th

week. Lipid profiling was done to determine the levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The LDL-C, HDL-C and (TC) estimations were done in auto-analyzer using GenX lipid parameter kits from Proton Biologicals and the (TG) was estimated in auto-analyzer using Coral clinical systems kit.

At the end of the study, the two control animals and three animals from the group 1 (standard) and group 2 (treatment) were sacrificed for collection of liver and aorta specimens for histopathology examination. Tissue sections of liver and aorta were preserved in 10% neutral buffered formalin later processed and stained using H&E stain. The slides were observed under microscope for fatty degeneration in liver and sub-intimal fat deposition in aorta.

Statistical Analysis

The changes in lipid parameters within the groups were analyzed using paired t-test to detect statistically significant difference. One way ANOVA with post-hoc Tukey test was done to evaluate the statistically significant difference between the treatment and standard groups. The results were expressed as mean \pm SD. A value of $p < 0.05$ was considered statistically significant. The histopathological changes were analyzed descriptively.

RESULTS

All the three groups exhibited a rise in mean TG after induction which was statistically significant compared to the baseline. Following treatment with teneligliptin, the mean TG was

Table 1. Comparison of mean \pm standard deviation of the body weight and lipid parameters after treatment (at the end of 6 weeks) between the three groups

Parameters	Group 1	Group 2	Group 3
Body weight (g)	267.62 \pm 18.91	258.37 \pm 37.20	324.33 \pm 19.66
Triglycerides (mg/dl)	46.21 \pm 16.05	57.51 \pm 16.00	77.25 \pm 10.08
Total cholesterol (mg/dl)	127.21 \pm 24.86	122.21 \pm 28.75	168.65 \pm 22.58
LDL (mg/dl)	44.96 \pm 7.61	40.70 \pm 10.62	62.90 \pm 4.49
HDL (mg/dl)	36.82 \pm 6.61	33.08 \pm 6.68	30.55 \pm 3.34

Table 2. Comparison of lipid parameters within groups using paired t test

Parameter	Group	Pair	Mean Paired difference	Std. Deviation	Std. Error Mean	p value*
Triglycerides	Group1	BaselineInduction	-54.20	10.83	4.42	<0.001
	Group1	Induction Treatment	24.71	18.11	7.39	0.021
	Group2	BaselineInduction	-39.51	8.92	3.64	<0.001
	Group2	Induction Treatment	28.70	7.61	3.10	<0.001
Total cholesterol	Group1	BaselineInduction	-181.80	28.64	10.82	<0.001
	Group1	Induction Treatment	132.95	23.57	8.90	<0.001
	Group2	BaselineInduction	-129.35	31.98	10.66	<0.001
	Group2	Induction Treatment	66.85	35.02	11.67	<0.001
LDL	Group1	BaselineInduction	-35.03	8.51	3.47	<0.001
	Group1	Induction Treatment	21.06	8.82	3.60	0.002
	Group2	BaselineInduction	-31.30	6.79	2.77	<0.001
	Group2	Induction Treatment	21.13	9.72	3.97	0.003
HDL	Group1	BaselineInduction	11.88	5.49	2.24	0.003
	Group1	Induction Treatment	-3.16	10.07	4.11	0.476
	Group2	BaselineInduction	14.05	5.21	2.12	0.001
	Group2	Induction Treatment	-1.01	9.11	3.72	0.796

* $p < 0.05$ is significant

57.51 ± 16.00 mg/dl while that in the simvastatin group was 46.21 ± 16.05 mg/dl (Table 1) and the mean reduction of TG (28.70 ± 7.61 mg/dl) in group 2 was statistically different ($p < 0.001$) as that of the mean reduction in group 1 (24.71 ± 18.11 mg/dl) with $p = 0.021$ (Table 2). However there was no statistical significance in the mean difference in TG levels between the two groups at the end of 6 weeks with $p = 0.318$ (Table 3).

Likewise, serum TC level which increased significantly due to induction with high cholesterol diet demonstrated a reduction following treatment both in standard as well as the treatment groups (Table 2). There was no statistically significant difference ($p = 0.923$) between the mean TC group 2 (122.22 ± 28.75 mg/dl) compared to that of group 1 (127.21 ± 24.86 mg/dl) after treatment (Table 1 & 3).

In addition, at the end of 6 weeks, serum LDL-C also revealed a parallel reduction in both groups with the mean of group 1 (44.96 ± 7.61 mg/dl) and 2 (40.70 ± 10.62 mg/dl) at the end of intervention being statistically significant compared to that of mean serum after induction with $p = 0.002$ & $p = 0.003$ for groups 1 and 2 respectively using paired t test (Table 1 & 2). A statistical significance in mean LDL-C levels could not be detected ($p = 0.735$) between the two groups using ANOVA (Table 3). However, a similar trend was not evident for serum HDL-C. A fall in HDL-C level could be noted in both groups with induction compared to the baseline. The mean HDL-C value after induction for group 1 (33.65 ± 4.41 mg/dl) and group 2 (32.06 ± 4.27 mg/dl) revealed a rise with the standard (36.82 ± 6.61 mg/dl) as well as

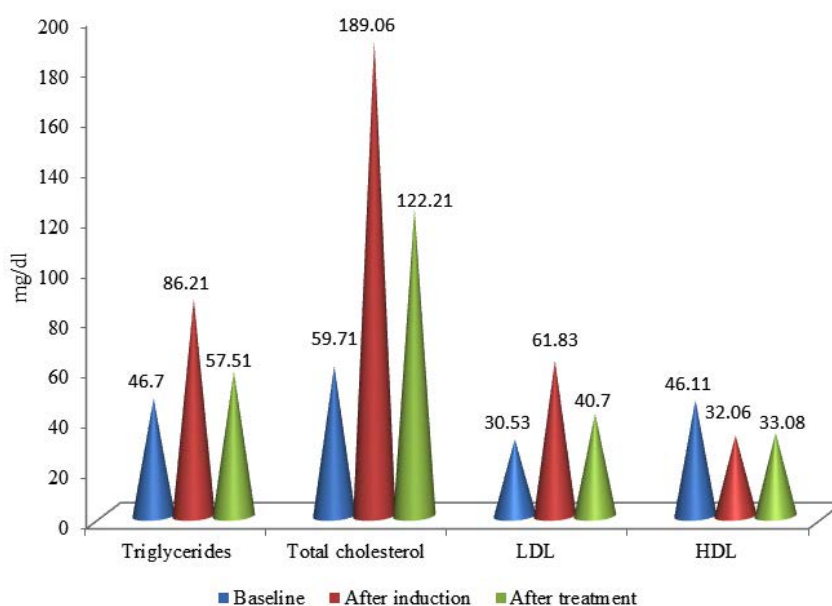


Fig. 1. Comparison of means of lipid parameters (mg/dl) of group 2 treated with teneligliptin

Table 3. Comparison between groups using one way ANOVA

Parameters	Mean difference between group 1 and 2	Standard error	Significance (p value)
Body weight(g)	9.25	14.67	.805
Triglycerides(mg/dl)	11.30	9.53	.318
Total cholesterol(mg/dl)	4.99	13.11	.923
LDL(mg/dl)	4.26	5.64	.735
HDL(mg/dl)	3.73	3.32	.516

* $p < 0.05$ is significant

the treatment drug (33.08 ± 6.68 mg/dl) but the increase produced by either of the drugs was not statistically significant with $p=0.476$ & $p=0.796$ for groups 1 and 2 respectively (Table 1 & 2).

Thus the net effect of teneligliptin evident was a statistically significant reduction in TC ($p<0.001$), TG ($p<0.001$) and LDL-C ($p=0.003$), but not the HDL-C ($p=0.796$) (Table 2 & Fig.1). A between group analysis using one way ANOVA revealed that there was no statistically significant difference between the groups treated with simvastatin and teneligliptin in all the four lipid parameters studied (Table 3).

Compared to baseline mean body weight of group 1 (273.75 ± 34.13 g) and group 2 (274.62 ± 37.20 g), there was a rise in body weight in both standard (286.62 ± 21.59 g) and treatment groups (280.87 ± 40.99 g) after induction though the increase was not statistically significant. In addition, a reduction in body weight was discernable in the study animals of both groups with treatment using standard/drug, yet the fall in mean body weight was

also not statistically significant in both groups. At the end of 6th week, the mean body weight of the standard group (267.62 ± 18.91 g) did not differ significantly ($p=0.805$) from that of the treatment group (258.37 ± 37.20 g) (Table 1 & 3).

Histopathologic examination using H&E stain revealed a normal liver section in groups 1 and 2 while mild fatty changes in the form of intracytoplasmic vacuolation with occasional areas of congestion could be detected in the control group. This indicates therapy with both simvastatin and teneligliptin had reversed the mild fatty change induced by the disease model. Sections of aorta on histopathology examination failed to reveal any significant changes in all three groups including the control group indicating that induction for a period of 4 weeks with high fat diet had not caused any significant atherosclerotic changes in the aorta of our study animals (Fig.2).

Fig.2 shows normal liver section in groups 1 and 2 while mild fatty changes in the form of intracytoplasmic vacuolation with occasional areas

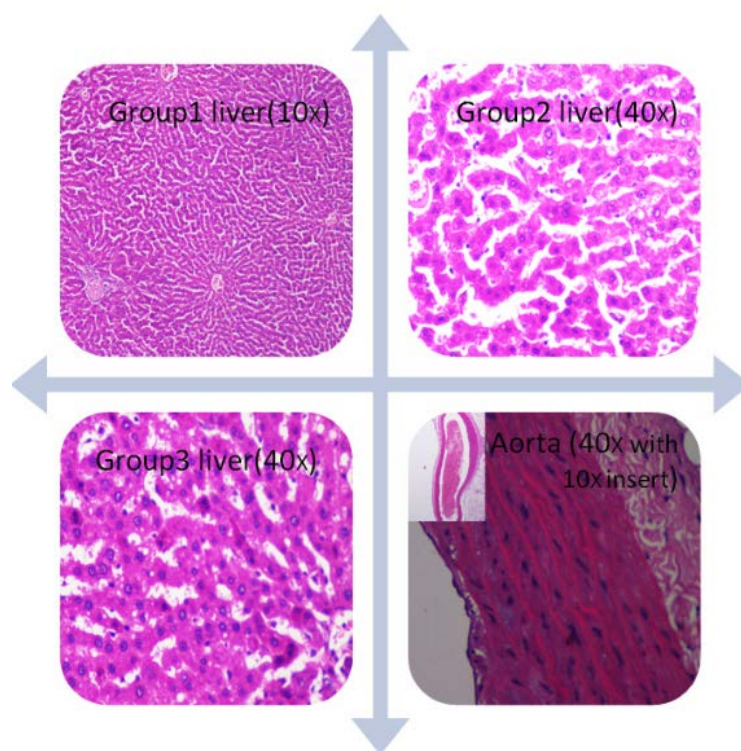


Fig. 2. Histopathological examination of the sections of liver under H&E staining

of congestion in the control group. Section of aorta of group3 reveals normal histology

DISCUSSION

This study which was done in a rat model of diet induced hyperlipidemia has explicitly brought out the lipid lowering property of teneligliptin. This study has demonstrated that there is no statistical significance in the reduction in blood lipids between simvastatin and teneligliptin including serum TC, TG, LDL-C. In addition, though the change in mean serum HDL-C revealed a greater rise with simvastatin compared to teneligliptin, the rise was not statistically significant in both groups implying that the beneficial effect of teneligliptin on HDL-C levels closely paralleled that of simvastatin though had lesser magnitude to be statistically significant.

Teneligliptin, classified as a class III DPP-4 inhibitor, has a unique structural feature that provides strong binding to DPP-4 enzymes compared with other gliptins and additional pleiotropic benefits⁶. The mechanism of action of teneligliptin which is used as a hypoglycemic agent in the treatment of T2DM is to amplify the levels of incretins namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide in response to meal intake, in turn resulting in augmented insulin secretion and attenuated glucagon secretion.^{6,7}

The hypolipidemic effect of teneligliptin is probably due to increased lipid mobilization from the adipose tissue and its oxidation by sympathetic activation facilitated through GLP-1 receptor activation, thereby reducing blood lipid levels as demonstrated by Boschmann *et al.*¹⁶

Also, the administration of teneligliptin has been shown to reduce the body weight in six-weeks-old C57BL/6N mice fed on a high-fat diet. The study has also established that teneligliptin increased oxygen consumption by 22% apart from proving that adipocyte hypertrophy and hepatic steatosis induced by a high-fat diet were suppressed by teneligliptin. In addition, a reduction in mean adipocyte size and hepatic TG content were demonstrable in this study.¹²

Another study investigating the effectiveness of teneligliptin in a mouse model of non-alcoholic steatohepatitis revealed that DPP4 inhibitors, by amplifying GLP-1 induced

AMPK activation in hepatocytes inhibits hepatic lipogenesis, thus consequentially inhibiting cholesterol and TG biosynthesis. The AMPK activation also promotes fatty acid β -oxidation and reduction of free fatty acid.¹⁴ Thus it is obvious that multiple pathophysiological mechanisms are potentially contributory to the hypolipidemic effect of teneligliptin.

However two clinical studies in Japanese population have yielded conflicting results on teneligliptin's effect on lipid profile. But both these studies were done in a small sample size which could be one of the reasons in discordance in their results.^{9,10} Yet another large clinical study done in a Korean population wherein about 1732 patients on any of the six other gliptins including alogliptin, gemigliptin, sitagliptin, vildagliptin, saxagliptin and linagliptin were switched over to teneligliptin, the effects of which were recorded at the end of 12 weeks. This study demonstrated that the weight decreased significantly by a mean of 0.4 kg from baseline in addition to a statistically significant decrease in the mean BMI by 0.1 kg/m². Among the serum lipid parameters, TC and LDL-C levels decreased from baseline to week 12 ($p < 0.05$). Though a favorable trend in the change from mean baseline values were noted for TG and HDL-C they were not statistically significant.¹⁷

The greater potency and additional beneficial effects of teneligliptin could be attributed to the fact that it binds more tightly to the DPP-4 enzyme compared to other gliptins because of the "J-shaped" structure formed by five rings.¹⁸ Thus the long-duration and strong binding property of teneligliptin could potentially yield greater clinically meaningful metabolic benefits.

CONCLUSION

This study has delineated the hypolipidemic activity of teneligliptin which will be of potential clinical value in the therapeutic use of teneligliptin in type 2 diabetic patients especially in the face of increased concomitance in the occurrence of T2DM and hyperlipidemia.

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