Evaluation of hyperglycaemic risk of atorvastatin: a dose dependent study on hyperlipidaemic rats

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ABSTRACT

Background: Statins (β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors) are the most prescribed medications worldwide to treat hyperlipidaemia with a proven ability to reduce major cardiovascular events. Recent data have revealed that statin therapy is associated with an increased risk for developing diabetes. The risk was most significant in patients taking atorvastatin, rosuvastatin and simvastatin.

Methods: Rats were divided into 3 groups, each comprising of 6 rats. Hyperlipidaemia was induced in all the animals after feeding with high fat diet for 15 days. Rats of groups 1, 2 and 3 were given atorvastatin 1.8 mg/kg (low-dose), 3.6 mg/kg (moderate-dose) and 7.2 mg/kg (intensive-dose) respectively orally for 60 days. 12 hours fasted blood samples were collected and analyzed for serum lipid profile, fasting blood glucose and HbA1c levels.

Results: The percentage increase in plasma blood glucose after 60 days of treatment in groups 1, 2, and 3 is 29.93%, 60.03% and 72.42% respectively and the variation in all the groups is statistically significant, p<0.0001. Regarding HbA1c values, the variation in low-dose group is statistically insignificant whereas the percentage increase in moderate-dose and intensive-dose groups is 19.45% (p<0.001) and 43.37% (p<0.0001) respectively.

Conclusions: In conclusion, there is significant increase in blood glucose and HbA1c levels leading to new-onset diabetes in both moderate-dose and intensive-dose groups. The risk is more in intensive-dose group when compared to moderate-dose group.

Keywords: Atorvastatin, HMG Co-A reductase inhibitors, Fasting blood glucose, HbA1c, New-onset diabetes

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of deaths in India.[1] The deaths due to CVD in India are more than 25% of all causes of mortality and are expected to contribute to more than half of the cases of heart diseases in the world within the next 15 year.[2] Atherosclerosis of coronary vessels is the main pathognomic mechanism responsible for CVD. Dyslipidemia is a major cardiovascular risk factor. Elevated plasma cholesterol levels have been shown to be major modifiable risk factor for atherosclerosis and thus present an important for intervention in primary prevention of CVD.[3]

The remarkable value of β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) in atherosclerotic CVD risk reduction is clearly established, based on landmark secondary intervention as well as primary prevention trials during the past two decades.[4,5] Various updated major guidelines have paved the way for greater attention to initiation and intensification of statin therapy in high-risk individuals (such as those with prior

CVD) and in individuals without CVD (such as those with diabetes and multiple risk factors).[6-8]

Now, statins are one of the most widely prescribed drug classes worldwide for lipid control and cardiovascular prophylaxis, and prescribing is continuing to grow. A study analyzed prescription data in India, during the period 2006-2009 and found that 8000 per 100,000 patients with CHD were receiving statins. Atorvastatin accounted for 80% of all prescriptions and was being produced by 62 manufacturers during that period.[9]

Recent studies indicating an increased incidence of diabetes associated with statin use are of concern. Although a small risk, given the extent of prescribing it could potentially result in a significant number of additional cases of diabetes per year. As a result of these reports, on February 28, 2012, the Food and Drug Administration added new safety label changes for the statins regarding the potential for increased hemoglobin A1c (HbA1c) and fasting plasma glucose.

There is sufficient evidence to support an association between statin use and new-onset diabetes but there are limited data to support a further increased risk of diabetes with intensive high-dose atorvastatin or simvastatin therapy.[10]

Atorvastatin is the most popular statin, more potent and has a much longer plasma half life than other statins.

Hence this study aims to evaluate the relationship between atorvastatin therapy and the risk of developing hyperglycaemia in a dose-dependent manner in hyperlipidaemic rats.

METHODS

The study was conducted at Central Animal House, Department of Pharmacology, Gandhi Medical College. Ethical clearance was obtained from Institutional Animal Ethics Committee meeting conducted on 7-3-2015 in Gandhi Medical College, Secunderabad before conducting the experiment (Reg. No:12/GMC/IAEC No.428/01C/CPCSEA/7th march 2015).

Animals

Animals were procured from Central Animal House, Department of Pharmacology, Gandhi Medical College, Secunderabad.

Healthy male albino rats of wistar strain 3-4 month old and weighing 150-250 g were used in the present study. The rats were inbred and grown under suitable laboratory conditions. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee, before conducting the experiment. They were housed in appropriately labelled steel cages according to groups (6 per cage) in a room maintained at 12 hour light-dark cycles and at a constant temperature of 24±2°C. The animals were provided with pellet chow and water ad libitum except during experimentation.

Drugs and chemicals

Cholesterol (Himedia Pvt Ltd, Mumbai), Deoxycholic Acid (Sigma-Aldrich Pvt Ltd, Mumbai), Tablet atorvastatin (Dr.Reddy's laboratory, Hyderabad) were used in the study.

Equipments

Feeding tube was used for the administration of the drug, glucometer was used for measuring blood glucose levels, capillary tubes were used for drawing blood from retro-orbital plexus and for glycated hemoglobin were estimated using HbA1c kit by ion exchange resin method.

Grouping

Eighteen male albino rats were randomised into groups. All rats were allowed a one-week acclimatization period to become accustomed to the laboratory conditions. Rats were randomly divided into three groups, each comprising six rats.

Duration of the study

The duration of the study was for 75 days and included 15 days of high fat diet feeding period and next 60 days of treatment period. Did not include days for acclimatisation, initial sample collection and dose selection.

Feeding period

All the rats were fed with high-fat diet for 15 days during the feeding period. Rats were supplied food water ad libitum.

Treatment period

Group I served as low-dose atorvastatin group & received atorvastatin 1.8 mg/kg/p.o. for 60 days, group II served as moderate-dose atorvastatin group & received atorvastatin 3.6 mg/kg/p.o. for 60 days and group III served as intensive-dose atorvastatin group & received atorvastatin 7.2 mg/kg/p.o. for 60 days.

Induction of hyperlipidemia

High cholesterol diet (HCD) comprised the following ingredients: cholesterol 5 g, deoxycholic acid 5 g, coconut oil 300 ml (300 g), and standard rat chow 700 g. Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow diet.[11]

Simultaneously cholesterol (5 g) was dissolved in 300 ml of warm coconut oil. This oil solution of cholesterol was

added slowly into the powdered mixture and thoroughly mixed to obtain soft homogenous cakes. These cakes were daily supplied to rats in each cage in sufficient quantities.

Atorvastatin dose selection

Method of preparation of atorvastatin suspension

The stock solution was prepared by dissolving 40 mg of atorvastatin in 70 ml of distilled water. The dose was selected using animal equivalent dose (AED) calculation for atorvastatin. Based on the respective body weight, doses were administered.

The daily dose of atorvastatin for albino rats was calculated by extrapolation from the human dose (20/40/80 mg/day) as described by M. N. Ghosh.

Blood sampling

All blood samples were collected within a one-hour period between 9:00 am and 10:00 am. Twelve hours fasted blood samples were collected under light ether anaesthesia by retro orbital puncture. Blood samples were collected on the day before the induction of hyperlipidaemia and on day 0, 30 & 60 during the treatment period. These blood samples were used for serum lipid analysis, fasting plasma glucose and HbA1c levels.

The various biochemical parameters in this study were estimated by following methods. Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein

(HDL-C) were determined by Endpoint colorimetric analysis. Low density lipoproteins (LDL-C), very low density lipoproteins (VLDL-C) were measured by using Friedwald formula. Blood glucose by glucose oxidase method. The estimation of HbA1c levels by HbA1c kit which follows ion exchange resin method.

Statistical analysis

Results were expressed as mean±standard deviation (SD) of six values (n=6) for each group. Statistical differences between the controls and the treatment groups were evaluated by using one way ANOVA (analysis of variance) followed by post-hoc Tukey's test and within group analysis by student paired t test.

Calculation of percentage change in serum parameters

Percentage change from initial values calculated by the formula.

Percentage change (%) on day 60 =

$$\frac{\text{day 0 serum levels} - \text{day 60 serum levels}}{\text{day 0 serum levels}}$$

RESULTS

Serum lipid analysis

Table 1 shows before (day 0) and after (day 60) treatment variations in lipid profile in each group of rats and ANOVA analysis.

Table 1: Comparison of serum lipid levels between different groups on day 0 (before treatment) and day 60 (after treatment).

Group	Treatment	Day 0				Day 60			
		TC	HDL	LDL	TG	TC	HDL	LDL	TG
I	Low-dose (atorvastatin 1.8 mg/kg)	134± 7.66	23± 1.26	80± 9.35	110± 8.19	87.67± 3.5	24.17± 0.75	53.33± 3.01	81.17± 2.78
II	Moderate- dose (atorvastatin 3.6 mg/kg)	135.3± 7.36	23.67± 1.96	75.17± 6.85	115± 9.75	74.33± 4.13	26.33± 1.03	41.67± 1.63	65.67± 3.55
III	Intensive- dose (atorvastatin 7.2 mg/kg)	134± 6.66	22.67± 1.21	72.5± 3.93	113.8± 10.36	68.67± 3.07	28.17± 1.72	30.33± 2.25	58.83± 4.26

Serum lipid levels are presented in mg/dl. Values are presented in mean±SD.

In group I, the TC value at day 0 was 134±7.66 mg/dl reduced to 87.67 mg/dl (mean±SD) at day 60 with p<0.0001. Percentage reduction is 34.57%. The HDL-C value on day 0 was 23±1.26 mg/dl increased to 24.17±0.75 mg/dl (mean±SD) at day 60 with p value insignificant. Percentage increase is 5.08%. The LDL-C value on day 0 was 80±9.35 mg/dl reduced to 53.33±3.01 mg/dl (mean±SD) at day 60 with p value<0.0001. Percentage reduction is 33.33%. The TG value on day 0 was 110±8.19 mg/dl reduced to 81.17±2.78 mg/dl (mean±SD) at day 60 with p<0.0001. Percentage reduction is 26.20%.

In group II, the TC value on day 0 was 135.3±7.36 mg/dl reduced to 74.33±4.13 mg/dl (mean±SD) at day 60 with p<0.0001. Percentage reduction is 45.06%. The HDL-C value on day 0 was 23.67±1.96 mg/dl increased to 26.33±1.03 (mean±SD) mg/dl at day 60 with insignificant p value. Percentage increase is 11.23%. The LDL-C value on day 0 was 75.17±6.85 mg/dl reduced to 41.67±1.63 mg/dl (mean±SD) at day 60 with p<0.0001. Percentage reduction is 44.56%. The TG value on day 0 was 115±9.75 mg/dl reduced to 65.67±3.55 mg/dl (mean±SD) at day 60 with p<0.0001. Percentage reduction is 42.89%.

In group III, the TC value on day 0 was 134±6.66 mg/dl reduced to 68.67±3.07 mg/dl (mean±SD) on day 60 with p<0.0001. Percentage reduction is 48.75%. The HDL-C value on day 0 was 22.67±1.21 mg/dl increased to 28.17±1.72 mg/dl (mean±SD) on day 60 with p value insignificant. Percentage increase is 24.26%. The LDL-C value on day 0 was 72.5±3.93 mg/dl reduced to 30.33±2.25 mg/dl (mean±SD) on day 60 with p<0.0001. Percentage reduction is 58.16%. The TG value on day 0 was 113.8±10.36 mg/dl reduced to 58.83±4.26 mg/dl (mean±SD) on day 60 with p<0.0001. Percentage reduction is 48.3%.

Blood glucose analysis

Table 2 shows variations in mean blood glucose levels on days 0, 30 and 60 in each group of rats and ANOVA analysis.

Table 2: Comparison of blood glucose levels between different groups on different days (0, 30 and 60).

Group	Day 0	Day 30	Day 60
I	78.5±15.07	89.33±11.43	102±4.05
II	74.17±10.09	97.83±9.30	118.7±5.68
III	80.5±14.90	112.8±9.74	138.8±8.61

Blood glucose levels are presented in mg/dl. Values are presented in mean±SD.

In group I, mean blood glucose levels increased from 78.5±15.07 mg/dl at day 0 to 89.33±11.43 mg/dl (mean±SD) at day 30 with insignificant p value. On day 60 the levels increased to 102±4.05 mg/dl (mean±SD) with p value<0.001. The percentage increase on day 60 is 29.93%. In group II, the mean blood glucose levels increased from 74.17±10.9 mg/dl at day 0 to 97.83±9.30 mg/dl (mean±SD) at day 30 with p<0.001. On day 60 the levels increased to 118.7±5.68 mg/dl (mean±SD) with p value<0.0001. Percentage increase on day 60 is 60.03%. In group III, the mean blood glucose levels increased from 80.5±14.9 mg/dl at day 0 to 112.8±9.74 mg/dl (mean±SD) at day 30 with p<0.0001. On day 60 the levels

increased to 138.8±8.61 mg/dl (mean±SD) with p<0.0001. Percentage increase on day 60 is 72.42%.

Table 3 shows before (day 0) and after (day 60) treatment variations of mean HbA1c levels in each group of rats and ANOVA analysis

In group I, mean HbA1c value varied from 5.25±0.36 at day 0 to 5.93±0.36 (mean±SD) at day 60 with no significant variation. In group II, mean HbA1c value increased from 5.86±0.45 at day 0 to 7.0±0.37 (mean±SD) at day 60 with p<0.001. Percentage increase is 19.45%. In group III, mean HbA1c value increased from 6.11±0.52 at day 0 to 8.76±0.77 (mean±SD) at day 60 with p<0.0001. Percentage increase is 43.37%.

Table 3: Comparison of HbA1c levels percentages (%) between different groups on day 0 (before treatment) and day 60 (after treatment).

Group	Day 0	Day 60
I	5.25±0.36	5.93±0.36
II	5.86±0.45	7.0±0.37
III	6.11±0.52	8.76±0.77

HbA1C levels are presented in percentages (%).

Table 4 shows variations in mean weights on days 0, 30 and 60 in each group of rats and ANOVA analysis.

In group I, the mean weights varied from 309.3±20.19 gms at day 0 to 318±15.53 gms (mean±SD) at day 30 and to 331.7±10.93 gms (mean±SD) at day 60 with no significant variation. In group II, the mean weights varied from 328±6.22 gms at day 0 to 341±8.67 gms (mean±SD) at day 30 with no significant variation. On day 60 the mean value increased to 356±11.9 gms (mean±SD) with p<0.001. In group III, the mean weights varied from 343.7±9.37 gms at day 0 to 358.8±9.23 gms (mean±SD) at day 30 with no significant variation. On day 60, the mean value increased to 374.3±8.77 gms (mean±SD) with p<0.001.

Table 4: Comparison of weights between different groups on different days (0, 30 and 60).

Group	Treatment	Day 0	Day 30	Day 60
I	Low-dose group (atorvastatin 1.8 mg/kg)	309.3±20.19	318.7±15.53	331.7±10.93
II	Moderate-dose (atorvastatin 3.6 mg/kg)	328±6.22	341±8.67	356±11.9
III	Intensive-dose (atorvastatin 7.2 mg/kg)	343.7±9.39	358.8±9.23	374.3±8.77

Weights are presented in gms.

On day 0: Post-hoc Tukey's multiple comparison test showed no significant difference in mean lipid profile values, blood glucose levels, HbA1C levels between the groups. Post-hoc analysis showed that group III mean weights differed significantly from group I mean weights.

On day 60: Post-hoc Tukey's multiple comparison test showed highly significant difference in mean TC, LDL

and TG values whereas no much variation in HDL values between the groups. Post-hoc Tukey's test showed no significant difference in mean blood glucose levels between groups I and II whereas group III showed significant variation from other two groups. Post-hoc Tukey's test showed significant difference in HbA1c levels between the groups. Post-hoc Tukey's test showed that group II mean weights and group III mean weights significantly differed from group I mean weights.

Table 5: Post-hoc Tukey's multiple comparison test.

Groups compared	Day 0	Day 60
Group I TC vs. group II TC	0.5	12.79***
Group I TC vs. group III TC	0.0	10.42***
Group II HDL vs. group III HDL	0.45	0.84
Group II HDL vs. group I HDL	0.30	0.99
Group III LDL vs. group I LDL	3.23	10.64***
Group III LDL vs. group II LDL	1.15	8.55***
Group I blood glucose vs. group II blood glucose	1.01	3.901
Group II blood glucose vs. group III blood glucose	1.48	4.72*
Group I blood glucose vs. group III blood glucose	0.46	8.62***
Group I HbA1c vs. group II HbA1c	3.03	5.25**
Group II HbA1c vs. group III HbA1c	1.23	4.35*
Group I HbA1c vs. group III HbA1c	4.26	13.95***
Group I weights vs. group II weights	3.84	5.00*
Group II weights vs. group III weights	3.22	3.77
Group I weights vs. group III weights	7.06***	8.78***

*p<0.01, **p<0.001, ***p<0.0001.

DISCUSSION

Atorvastatin is the most popular statin, more potent and appears to have the highest LDL-C lowering efficacy at maximal daily dose of 80 mg. At this dose a greater reduction in TGs is noted if the same was raised at base line. Hence Atorvastatin has been selected for the present study.

In the present study the dose dependent hyperglycemic risk of Atorvastatin was evaluated in hyperlipidemic rats. The rats were divided into 3 groups, group I (low dose group), group II (moderate dose group) and group III (intensive dose group) which received atorvastatin in the doses equivalent to human doses of 20, 40 and 80 mg respectively. Serum lipid profile and HbA1c were analyzed at days 0 and 60. Changes in weights and blood glucose levels were analyzed at days 0, 30, and 60.

In the study the mean blood glucose levels in low dose group did not very much on day 30 but the levels significantly varied on day 60. In moderate and intensive dose groups, the levels varied significantly on day 30 and on day 60 the variation was highly significant. The percentage increase was 60.03% and 72.42% in groups II and III respectively.

According to the studies done by Preiss et al and Waters et al, intensive dose statin therapy was associated with an increased risk of new onset diabetes compared with moderate dose statin therapy.[12] In supporting the above studies the present study showed that intensive dose group showed 72.42% increase in blood glucose levels were as moderate dose group showed 60.03% increase in blood glucose levels.

HbA1c levels in low-dose group did not vary much on day 60. Moderate-dose group showed significant variation on day 60. The percentage increase was 19%. Intensive-dose group showed highly significant variation on day 60. The percentage increase was 43%.

In conclusion, there is significant increase in blood glucose and HbA1c levels in both moderate-dose and intensive-dose groups. The increase is more in intensive-dose group when compared to moderate-dose group.

Despite the increased risk of new onset diabetes in susceptible individuals studies clearly show a benefit of statins in reducing major cardiovascular events.[13] A meta-analysis reported that treating 255 patients with statins for 4 years would induce 1 case of diabetes, but in the meantime, it would prevent 5.4 coronary deaths or myocardial infarctions for each millimolar reductions in serum LDL-C. In support of the above studies, the present study showed a highly significant reduction in LDL levels in all the groups i.e., low dose, moderate dose and intensive dose groups with percentage reductions of 33%, 44.5% and 58% respectively.

The overall benefits of statins strongly outweigh any risk, including in those at risk of diabetes. Moreover the strongest predictors of whether a patient will develop diabetes (regardless of whether he /she takes a statin) still include older age, increased weight and higher blood sugar levels before statin use. Statins may be simply unmasking diabetes mellitus that would have developed any way based on these other very important risk factors.[14]

It remains important to increase exercise as tolerated, make healthy food choices and to lose excess weight if one has or is at risk for diabetes mellitus. Steps should be

taken to ascertain patients who are at risk by monitoring them both clinically and biochemically according to national guidelines; to identify the onset of new onset diabetes and manage the condition appropriately.

CONCLUSION

The present study evaluated the dose-dependent relationship between atorvastatin therapy and the risk of hyperglycemia. There is significant increase in blood glucose levels in both moderate-dose and intensive-dose groups. The increase is more in intensive-dose group when compared to moderate-dose group. There is significant increase in HbA1c levels in moderate as well as intensive- dose groups. There is highly significant dose-dependent reduction in TC, LDL-C and TG levels. There is little effect on HDL-C levels.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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