Effect of Atorvastatin on Lipid Profile and Mevalonic Acid Levels, a Biomarker in North Indian Normocholesterolemic Subjects

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ABSTRACT: Objective: To evaluate the effect of atorvastatin on plasma lipid levels, plasma and urine Mevalonic acid (MVA) levels following 7-day treatment in healthy, adult, human, male subjects from North India. Methodology: The study was carried out in 12 healthy volunteers. The subjects were randomly divided into two groups. The group 1 (n=8), received atorvastatin 20 mg and group 2 (n=4) received matching placebo once daily for 7-days. Blood sample for analysis were collected at baseline (day-0) and the end of the study (day-7). Results: Atorvastatin group showed significant reduction (p < 0.05) in total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) levels as compared to the basal levels. There was also significant reduction in plasma levels of triglycerides (TG), and very low-density lipoprotein-cholesterol (VLDL-C) in treatment group when compared with placebo group. High-density lipoprotein-cholesterol (HDL-C) levels did not show appreciable change in both the groups as compared to basal levels. Plasma MVA levels and AUC0-24h were significant lower on day-7 compared to day-0 (p < 0.05) and were correlated well with significant reduction in urinary MVA excretion. Large inter-individual variability in lipid and MVA profile was observed prior to and post 7 day treatment in both groups akin with published reports. Conclusions: Atorvastatin was safe and well tolerated following once daily 20 mg dose for 7-days and significantly reduced MVA and lipid levels in healthy, adult, North Indian volunteers. Measurement of MVA in plasma and urine can be used as a surrogate marker of statin efficacy in early development and comparative clinical studies.

KEY WORDS: Cholesterol; Statin; Atorvastatin; Mevalonic acid.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by marked elevation in TC and LDL-C. FH is a risk factor for cardiovascular mortality and morbidity worldwide and may lead to atherosclerosis. It is well recognized that lipids and family of lipoproteins play a significant role in the formation and progression of atherosclerotic disease. Achievement of cholesterol goals is an important objective of lipid lowering therapy in clinical practice and a challenging task [JAMA 2001].

Hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase catalyses the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol synthesis (Parker TS et al., 1984; Abrar M and Martin PD, 2002; Jemal MA et al., 2000). HMG-CoA reductase inhibitors (statins) are widely used in treating adults with primary hypercholesterolemia in whom the LDL-C levels are increased, especially in patients with FH. Pharmacological inhibition of HMG-CoA reductase leads to up-regulation of LDL receptors, thus increasing the rate of removal of LDL-C from plasma and thereby reducing its level.

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Atorvastatin, a synthetic lipid-lowering agent, is selective competitive inhibitor of HMG-CoA reductase (Bakker-Akrema R et al., 1996; Bernini F et al., 2001; Cilla DD et al., 1996). It lowers the plasma cholesterol levels by inhibiting endogenous cholesterol synthesis. Administration of atorvastatin (10-80mg), once daily for 6 weeks has been shown to reduce LDL-C by 25%-61% in dose response studies in hypercholesterolemic patients and has also been shown to reduce TG by up to 32% in hypercholesterolemic patients (Navrocki JW et al., 1995). Available literature reveals that plasma concentrations following oral administration of statins correlate poorly with the clinical efficacy of these drugs (Lennermas H and Fager G, 1997). Hence, it is difficult to build a correlation between pharmacokinetics and pharmacodynamic effect of these drugs. Also, there is considerable inter-individual variability in response to the statin therapy (Frans H et al., 2001).

Elevation of blood cholesterol can result from several different biochemical mechanisms including, inordinately high cholesterol absorption in the intestine, high endogenous biosynthesis of cholesterol, and poor metabolism of cholesterol to bile acids. Measuring cholesterol levels in blood cannot differentiate between these three mechanisms. Measurement of additional metabolites/biomarker is required to identify the site of action of these lipid-lowering agents.
MVA is synthesised by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (Parker TS et al., 1984) and is a precursor for cholesterol. Plasma mevalonate levels correlate well with hepatic HMG-CoA reductase activity in rats and humans (Parker TS et al., 1984; Yoshida T et al., 1993; Saini GS et al., 2006) and are related to the rate of whole-body cholesterol synthesis (Parker TS et al., 1984). Plasma MVA concentrations can therefore be used as a biomarker of HMG-CoA reductase inhibition by the statin class of drugs and thus as a measure of statin activity.

Estimation of plasma MVA concentrations has been shown to be a good indicator of in vivo cholesterol biosynthesis (Parker TS et al., 1984; Naoumova RP et al., 1997; Martin PD et al., 2002). There is evidence that following single dose treatment with HMG-CoA reductase inhibitors like pravastatin, simvastatin, atorvastatin and rosuvastatin, there is a reduction in plasma levels and urinary excretion of MVA (Lindenthal B et al., 1996). Also, plasma MVA levels have been shown to correlate closely with HMG-CoA reductase activity in human liver (Yoshida T et al., 1993). Statins can be compared head-to-head early in drug development (Phase I), by estimating effects on MVA concentrations in plasma and urine. Recent clinical studies have started using MVA measurements in biofluids as an alternative to conventional sterol balance studies in assessing human cholesterol synthesis (Frans H et al., 2001; Saini GS et al., 2006). Taken together, these reasons support the use of MVA levels in biofluids as a useful biomarker for cholesterol biosynthesis. Such measurements, done in early clinical trials, potentially provide a useful decision making tool in drug development.

Statins are widely used as lipid lowering therapy in India, but there are no published reports on use of MVA as biomarker. In the present study, the use of MVA in addition to lipid profile has been explored as early biomarkers of statin (atorvastatin) efficacy, in North Indian population. Due to high inter-individual variability, and population variability to statin therapy, there is a need to evaluate or monitor the therapy using additional biomarkers.

Methodology

Study design

The study was conducted as open label, randomized, placebo controlled, parallel pharmacodynamic pilot study to evaluate the effect of multiple doses of atorvastatin (20 mg) on the baseline plasma and urine MVA levels and plasma lipid levels in healthy, adult, Indian, male, human volunteers. The order of receiving the treatments for each subject during the study was as per randomization schedule, generated using SAS (version 9.1.3, SAS Institute NC, USA).

Study medications

The subjects received an oral dose of either atorvastatin 20 mg (STORVAS 20mg tablets each containing 20mg of atorvastatin calcium, manufactured by Ranbaxy Laboratories Limited, India) or matching placebo with 240 ml of drinking water for 7-days during the study period. Subjects were advised to avoid diets rich in fat and cholesterol during the trial to minimize any potential dietary effects on lipid levels. The subjects were required to fast over night (10-12 hrs) before the morning dose/sample on day-0 and day-7 (urine and plasma samples) and day 1 and then on day-8 (post 7-days treatment) for lipid profile samples.

Study subjects

Twelve healthy, adult, male subjects between 18 - 42 years participated in the study. The subjects weight (kg) and height (cm) ranged from 48-68 and 156-176, respectively. All were in good health as evidenced by their medical histories, physical examinations and laboratory tests performed within 28-days, prior to commencement of the study. None had history of any allergy to atorvastatin and related compounds. Subjects did not receive any medication during the two weeks period prior to the start of the study. They were instructed during screening not to take any prescription and OTC medications subsequently until the completion of the study. All the subjects abstained from any xanthine containing food or beverages or alcoholic products for 48 hours prior to dosing and throughout the sampling schedule during each period. Subjects were admitted and housed in Ranbaxy Clinical Pharmacology Unit (RCPU), Delhi from 12 h before dose and were discharged 24 hours after dose administration on day-0 and day-7.

Blood sampling

The baseline blood samples were collected on day-0 (before dosing) at 0, 2, 4, 8, 12, 16 and 24 hours time interval from forearm vein using an indwelling catheter into vacutainers containing EDTA as anticoagulant. The blood samples were again collected at pre-dose and at 2, 4, 8, 12, 16 and 24 hours post-dose on day-7. Blood sample (4 ml) for determination of lipid levels was collected in morning after an overnight fast of at least 10 hours, in on day-1 (Pre treatment) and then on day-8 (post 7-days treatment). For each subject, a total of 16 blood samples were drawn during the entire study period. Blood sampling was done on day-0 (baseline), and then on day-7. After collection, the blood samples were centrifuged to separate the plasma. All plasma samples were stored below -70°C until analysis.
Urine sampling

The urine samples were collected on day-0 (baseline) and day-7 at (- 4-0h) and at 0-4, 4-8, 8-12, and 12-24 hour intervals. For MVA estimation, day-0 and day-7 represent pretreatment and post treatment days. All urine samples were stored below -70°C until analysis.

Ethical considerations

The study was conducted at Ranbaxy (RCPU) according to a protocol approved by Jamia Hamdard Institutional Review Board. This research was carried out in accordance with the clinical research guidelines defined in the U.S. 21 CFR part 312.20 and the principles enunciated in the declaration of Helsinki (South Africa 1996). All the subjects provided written informed consent before entering the study.

Analytical procedures

Estimation of TC, TG and HDL-C

TC, TG and HDL-cholesterol were measured in the plasma by cholesterol flex reagent cartridge (Dade Behring, USA). It is an in vitro diagnostic test used for quantitative determination of TC in plasma. The test was performed on the Dimension clinical chemistry system after the estimation method was validated and the instrument was calibrated. Sampling, reagent deliver, mixing, processing and printing of the results were automatically performed by the Dimension system. Estimation of LDL-C LDL-C was calculated by the Friedewald formula (Friedewald WT et al., 1972): \[\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{1}{5} \times \text{TG}\]

Bioanalytical method for urine and plasma MVA

Plasma and urine MVA concentrations were quantified by a validated LC-MS/MS method developed at Metabolism and Pharmacokinetics Department, Ranbaxy Research Laboratories (Saini GS et al., 2006). The calibration range was 0.5 ng/ml to 50 ng/ml. Quality control samples were prepared by taking into consideration the endogenous MVA levels. MVA was extracted as mevalonolactone (acidic pH) from plasma using solid phase extraction procedure and was converted to acid form (basic pH) and read as m/z 147/59. D-7 mevalonolactone was the internal standard and read as 154/59. The concentrations were read using analyst software, version 1.4.

Data analysis

WinNonlin 4.1 professional software (Pharsight Corporation, USA) was used to calculate plasma MVA area under the curve from 0 to 24 hour (AUC0-24h) for treatment and placebo groups. AUC’s were calculated using trapezoidal rule. Plasma MVA AUC0-24h on day-7, following 7-day treatment with atorvastatin or placebo was compared to that obtained on day-0 (baseline). The plasma MVA levels were converted to percentage with respect to zero hour MVA concentration on both day-0 and day-7, due to high variability observed in baseline MVA levels.

Statistical analysis was performed using SAS (version 9.1.3, SAS Institute NC, USA). The repeated measures for intra-group variance “ANOVA” were tested by the application of F-test. Besides intra-group, paired comparisons was done using students paired t-test. Furthermore, student’s t-test was used for measuring difference between the atorvastatin treated and placebo groups. A confidence interval of 95% was used. For all statistical analysis p < 0.05 was considered significant.

Results

Pharmacodynamic effect of atorvastatin was evaluated in present study following 20 mg once daily treatment for 7-days in healthy North Indian subjects. The demographics of the subjects are given in Table 1. Marked reduction in TC, LDL-C, VLDL-C, TG, plasma and urine MVA levels was observed following atorvastatin treatment (Table 2). The lipid profile at baseline and post treatment is compared in Fig. 1.

In atorvastatin treatment group the mean reduction in TC levels was 27.49 %, (p < 0.05) compared to baseline levels. Likewise the reduction in plasma TG, LDL-C and VLDL-C was 37.54, 33.86 and 37.61%, respectively. Plasma HDL levels did not show appreciable change after 7-days treatment. The change in all these parameters except HDL was significant (p > 0.05) as given in Table 2. On the contrary, a slight increase in mean plasma lipid levels (TC, TG, LDL-C and VLDL-C) following 7-day treatment with placebo.

Table 1. Demographics of the subjects (n = 12).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.08 ± 7.93</td>
<td>18 - 42</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.25 ± 6.85</td>
<td>48 - 68</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.33 ± 6.37</td>
<td>156 – 176</td>
</tr>
</tbody>
</table>
Table 2. Plasma lipid concentrations, MVA exposure and urinary MVA output in healthy subjects, prior to (baseline) and after 7-daue treatment with placebo / atorvastatin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Treatment</th>
<th>% Reduction with respect to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (Baseline)</td>
<td>Mean ± SD (Post-Dose)</td>
<td>Mean ± SD (Baseline)</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>117.50 ± 27.20</td>
<td>128.00 ± 28.46</td>
<td>145.5 ± 34.20</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>84.25 ± 40.12</td>
<td>90.50 ± 49.41</td>
<td>115.87 ± 56.57</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>30.50 ± 6.35</td>
<td>30.50 ± 3.69</td>
<td>34.00 ± 3.74</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>70.15 ± 23.82</td>
<td>79.40 ± 20.30</td>
<td>88.30 ± 24.76</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>16.85 ± 8.03</td>
<td>18.10 ± 9.88</td>
<td>23.20 ± 11.34</td>
</tr>
<tr>
<td>Plasma AUC0-24h (% MVA*h)**</td>
<td>3631.20</td>
<td>3024.70</td>
<td>3033.00</td>
</tr>
<tr>
<td>Urinary MVA output (µg)</td>
<td>962.50 ± 277.70</td>
<td>942.60 ± 135.80</td>
<td>1096.90 ± 397.10</td>
</tr>
</tbody>
</table>

* statistically significant decrease following 7-day treatment (p < 0.05).

** AUC is a single value, obtained from mean MVA plasma levels.

Fig. 1 Lipid Profile on day-0 and day-7 days atorvastatin (20 mg) treatment in healthy subjects (n = 8).

The plasma MVA levels depicted a diurnal rhythm on all occasions as reported earlier by other investigators (Frans H et al., 2001; Naoumova RP et al., 1997). The baseline concentrations on day-0 for all 12 subjects are plotted to depict the diurnal rhythm in normocholesterolemic subjects (Fig. 2).

High inter-individual variability in the MVA baseline levels was observed in both groups. But on careful inspection of the individual profiles, the effect of atorvastatin on MVA levels is clearly evident. To offset the variability in plasma MVA baseline levels on day-0 and day-7, the plasma MVA concentrations were transformed as percentage with respect to 0 hour concentration (Fig. 3). MVA levels exhibit significant reduction following 7-days atorvastatin administration. The plasma MVA AUC0-24h decreased by 31.43 % as compared to the baseline MVA AUC0-24h (Table 2, Fig. 3).
Fig. 2 Baseline MVA levels in plasma of healthy subjects over 24 hour period depicting the diurnal rhythm (n = 12).

Fig. 3 Plasma MVA levels in healthy subjects prior to (baseline) and after 7-days treatment with 20 mg once daily dose of atorvastatin (n = 8).

The urine output of MVA following 20mg daily dose of atorvastatin for 7-days reduced by 25.3% following 20 mg daily dose of atorvastatin for 7-days. The fall in MVA output was significant as compared to the baseline levels (p < 0.05).

**Discussion**

Cholesterol levels in blood have been studied for decades because of its association with heart disease. The absolute levels of cholesterol in blood provide a quantitative estimate of cardiovascular disease risk for an individual. Measuring additional biomarkers in biofluids such as plasma and urine provide an indication of rate of cholesterol synthesis. Statins have been the first line of drugs for the treatment of hypercholesterolemia inspite of high pharmacodynamic variability. This variability has been explained with simultaneously measured MVA, along with other known biochemical parameters (Frans H et al., 2001).
Several reasons support the need to evaluate or monitor the efficacy of statin therapy by measuring an intermediate step of cholesterol synthesis. First, plasma concentrations following oral administration of statins correlate poorly with the clinical efficacy of these drugs. Hence, it is difficult to build a correlation between pharmacokinetics and pharmacodynamic effect of the statins. Second, there is well documented inter-individual and population variability to statin therapy. In some clinical trials, biochemical parameters such as mRNA levels of HMG-CoA reductase in leukocytes, MVA levels in biofluids such as plasma and urine, have been determined in addition to lipid levels (Frans H et al., 2001). Third, there is evidence that following 6-8 week treatment with HMG-CoA reductase inhibitors like pravastatin, simvastatin and atorvastatin, there is a reduction in plasma concentrations and urinary excretion of MVA (Naoumova RP et al., 1997; Naoumova RP et al., 1996). Statins can be compared head-to-head and proof of concept can be obtained early in drug development (Phase I), by estimating effects on MVA concentrations in plasma and urine. Fourth, other investigators have found that levels of plasma and urine MVA are a good indicator of the \textit{in vivo} rate of cholesterol biosynthesis (Abrar M and Martin PD, 2002; Jamal MA et al., 2003; Frans H et al., 2001). Recent clinical studies have started using MVA measurements in biofluids as an alternative to conventional sterol balance studies in assessing human cholesterol synthesis. Taken together, these reasons support the idea that levels of MVA in biofluids represent a useful biomarker for cholesterol biosynthesis. Such measurements, done in early clinical trials, potentially provide a useful decision making tool in drug development.

In several 6-week dose-ranging trials with atorvastatin report significant reduction in LDL-C levels from baseline (reduction in LDL-Cholesterol was: 38% to 54%) in a 165 patient study receiving 10-80 mg of atorvastatin (Schneck DW et al., 2003). In another study, 185 patient receiving 10-40 mg of atorvastatin produced greater reduction in LDL-C (38% to 51%) than the respective doses of simvastatin, pravastatin, lovastatin, and fluvastatin [Jones P et al., 1998]. A reduction of 25% to 61% in plasma LDL-C levels was produced with atorvastatin 2.5-80 mg in 81 patients, when compared to a six-week placebo controlled study (Nawrocki JW et al., 1995). After atorvastatin 10-80 mg (n = 641), rosuvastatin 10-40 mg (n = 643), simvastatin 10-80 mg (n = 655) and pravastatin 10-40 mg (n = 492) administration, significant reduction (p < 0.001) in LDL levels was observed. LDL-C levels decreased by 37% to 51%, 46% to 55%, 28% to 46% and 20% to 30% after atorvastatin, rosuvastatin, simvastatin and pravastatin administration, respectively in the Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin (STELLAR) (Jones PH et al., 2003).

Measurement of plasma MVA or urinary excretion of MVA has been demonstrated as to be good indicators of the \textit{in vivo} rate of cholesterol biosynthesis. HMG-CoA reductase inhibitors decrease plasma concentration and urinary excretion of MVA [Parker TS et al., 1984; Abrar M and Martin PD, 2002; Naoumova RP et al., 1996; Goldstein JL and Brown MS, 1990; Woollen BH et al., 2001]. There are reports that in FH patients in South Africa, treated with high doses of atorvastatin, simvastatin or pravastatin, poor responders had a lower basal level and a lesser reduction in plasma MVA, as compared with good responders [Frans H et al., 2001]. There was significant reduction in plasma and urine MVA levels following rosuvastatin treatment for 14-days [Naoumova RP et al., 1997].

In conclusion, the present clinical study demonstrates the effect of atorvastatin (20 mg) on plasma, urine MVA levels and lipid profile in normcholesterolemic subjects from North India. There was significant reduction in plasma MVA AUC0-24h, which is translated into significant decrease in urinary MVA output with 7 days treatment. A high inter-individual variability is also seen in this study, consistent to that reported by other researchers [Frans H et al., 2001; Kajinami K et al., 2004; Kajinami K et al., 2005]. Taken together, measurement of MVA in biofluid can be used as a biomarker for statin therapy and as a screening tool during early drug development. The use of MVA as surrogate marker for statin therapy / development should be further evaluated under controlled conditions in diverse ethnicity involving larger population to draw substantial conclusions.

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References


