Antihyperlipidemic and antiatherogenic activity of simvastatin may involve modulation of the expression of lecithin:cholesterol acyl transferase

Adeniran Sanmi Adekunle∗, John Olabode Fatoki and Temitope Isaac Adelusi

Department of Biochemistry, Ladoke Akintola University of Technology, Nigeria

Introduction: The statin-induced effects on high density lipoprotein (HDL) are relatively small compared with those of low density lipoprotein (LDL) and, as a result, most clinical trials of statins are underpowered with respect to HDL parameters. This study experimentally investigated, the effects of statin on serum lipids, atherogenic index and examined the possibility of a relationship amongst serum concentrations of HDL-C, atherogenic index and activity of lecithin:cholesterol acyl transferase. Method: Thirty albino rats equally divided into 2 groups were used for the study. Group 1 was given 0.05mg/g of statin daily for 28 days, while group 2 served as control. HDL concentration was determined as a measure of HDL-C. Total cholesterol (TC), triglyceride (TG) and HDL-C were determined spectrophotometrically while LDL-C was calculated using the Friedewald formula. Effect on the activity of lecithin:cholesterol acyl transferase was determined by the difference between the amount of free cholesterol converted to cholesteryl ester in the two experimental groups. Effects on body and relative organs weights were also determined. Results: The administration of statin caused a significant increase in serum concentration of HDL-C, while levels of LDL-C, triglyceride and total cholesterol were reduced. Simvastatin caused a significant reduction in the atherogenic index (TC/HDL-C; LDLC/HDL-C). The administration of statin significantly induced the activity of lecithin:cholesterol acyl transferase (LCAT) as evident by reduced serum concentration of free cholesterol when compared with control. The administration of statin caused reduced body and relative organs weights. Conclusion: The study showed that serum antihyperlipidemic and antiatherogenic activity of statin may involve the induction of LCAT.

Key words: atherogenicity, statin, lipids, lipoproteins, lecithin : cholesterol acyl transferase

Received: 09 May, 2013; revised: 02 September, 2013; accepted: 19 September, 2013; available on-line: 22 November, 2013

INTRODUCTION

The use of statins has revolutionized the management of people at risk of having a cardiovascular event. Several effects of statins have the potential to reduce cardiovascular risk, with compelling evidence that the statin-induced reductions in LDL cholesterol (LDL-C) are implicated (Baigent et al., 2005; Sipahi et al., 2006). Currently, six different statins (simvastatin, pravastatin, lovastatin, fluvastatin, rosuvastatin and atorvastatin) are approved for treatment of hypercholesterolemia in humans (Brown, 2001). Despite differences in their pharmacokinetic profiles, all statins have at least one characteristic in common: they block the conversion of HMG-CoA to mevalonic acid with consecutive attenuation of the biosynthesis of cholesterol, which is associated with a reduction in serum total and low-density lipoprotein (LDL) cholesterol of as much as 20–31% and 28–42% during chronic treatment (Andrew et al., 2001). Because of these properties, statins have become the most widely prescribed lipid-lowering drugs to patients with elevated serum cholesterol levels. The discovery of statins led to important improvements in the primary and secondary prevention of coronary artery disease (CAD). Initial studies explored the impact of statin therapy on CAD progression and regression. Although the angiographic changes in response to therapy were modest, the accompanying clinical benefit appeared significant. Subsequent large prospective clinical trials have provided unequivocal evidence that cholesterol-lowering therapy with this class of compounds not only reduces the major coronary event rate in primary and secondary prevention, but also...
reduces all-cause mortality in secondary prevention. Several large trials demonstrated that statins are not only safe and well-tolerated but also significantly decrease the cardiovascular morbidity and mortality in hypercholesterolemic patients in both primary and secondary prevention (Shepherd et al., 1995; Sacks et al., 1996; Down et al., 1998). However, the striking benefit achieved with statin treatment in patients with a wide range of cholesterol levels, which cannot be attributed to their cholesterol lowering effect alone, has raised the question about the possible presence of additional effects of statins beyond their impact on serum cholesterol levels. Indeed, in recent years a substantial quantity of data has accumulated a notion that statins exert various effects on multiple targets. Studies have shown that statin inhibits the biosynthesis of isoprenoids which are essential for the posttranslational modification of several proteins involved in important intracellular signaling pathways (e.g. small GTP-binding proteins Ras and Rho). Another pathway affected by statins seems to be the regulation of the activity of the enzyme cholesteryl ester transfer protein (CETP), which transfers cholesteryl ester to very-low-density lipoprotein (VLDL) and LDL (Bruce & Tall, 1995). This study thus sought to evaluate the regulation of lecithin:cholesterol acyl transferase by simvastatin in experimental animal model.

METHODS

Materials. Simvastatin drug was purchased from a pharmacy in Ogbomoso, while the kits for measurement of lipids and lipoproteins were LABKIT products of CHEMILEX, SA (Barcelona, Spain). All other chemicals used including solvents are of analytical grade.

Study design. Thirty adult Wistar rats with average body weight of 190 grams were purchased and housed in well-ventilated wire cages to acclimatize for few days. They were fed with regular pelleted animal feed and were given unrestricted access to clean water. The protocol conforms to the guidelines of the National Institute of Health (NIH) (NIH publication 85–23, 1985) for laboratory animal care and use. Animals were randomly divided into two groups of fifteen animals each. Rats in group 1 were orally administered simvastatin (0.05 mg/g) per rat per day for 28 consecutive days. Group 2 served as control (received drug-vehicle, water). On day 29, the rats were sacrificed by cervical dislocation. The rats of the body and organs weight (mg).

Determination of biochemical parameters. Analyses of lipids and lipoprotein. Total cholesterol, triglyceride, and HDL-C were analyzed using spectrophotometric methods, while LDL-C was calculated. Total cholesterol concentration in the serum was determined spectrophotometrically using the cholesterol oxidase method at 546 nm, 37°C (Allain et al., 1974). HDL-C was determined by the spectrophotometric methods of (Assman et al., 1983) at 500 nm, 37°C. Triglycerides were determined using the spectrophotometric method (Bucolo & David, 1973). Low density lipoprotein cholesterol was calculated using the Friedwald formula (Friedwald et al., 1972).

Estimation of atherogenicity. The atherogenic index was estimated by calculating TC: HDL-C and LDL-C: HDL-C ratios.

Lecithin:cholesterol acyl transferase assay method. The activity of LCAT was assayed in serum using the method of Hitz et al., (1983). Briefly, a pool of serum was heated at 56°C for 30 minutes to inactivate the LCAT. The inactivated serum was incubated at 4°C for 15 minutes with 0.2% dextran sulfate, which caused the elimination of two-thirds of the lipoproteins (LDL+VLDL). This was sedimented by centrifugation at 1750×g for 15 minutes. The supernatant containing HDL was used as the substrate. A sample of the substrate (0.6 ml) was mixed with 1.0 ml of isopropyl alcohol while the remaining mixture was incubated at 27°C for 90 minutes. The precipitate was removed by centrifugation and the supernatant was taken for the estimation of free cholesterol present in the test sample at zero time. After 90 minutes, a 0.2 ml sample of the incubated mixture was mixed with 1.0 ml of isopropyl alcohol and the remaining mixture was incubated at 27°C for a further 90 minutes. At the end of 180th minutes, 0.2 ml of the incubated mixture was treated with 1.0 ml of isopropyl alcohol to arrest the reaction. The precipitated protein in all of the tubes were separated by centrifugation and the free cholesterol content in the supernatant was estimated. Control tubes containing only the substrate were treated similarly to check for the complete inactivation of serum during substrate preparations. LCAT activity was expressed as a function of the disappearance of free cholesterol during the incubation period.

Statistical analysis. Values were expressed as mean ± S.D. (standard deviation). Differences between groups were statistically compared using the Student’s t-test. The results were considered statistically significant for p<0.05.

RESULTS

Table 1 depicts the effects of simvastatin on serum lipid profile. The administration of simvastatin caused a significant increase and decrease in serum concentrations of HDL-C and free cholesterol respectively (p>0.05). Though not statistically significant, simvastatin reduced serum concentrations of total cholesterol, triglyceride and LDL-C. Simvastatin caused a significant reduction in the atherogenic index (TC: HDL-C and LDL-C: HDL-C ratios) (p<0.05). Table 2 shows the effects of the administration of simvastatin on body and organs weights. Simvastatin did not cause any appreciable reduction in the body and organs weight (p<0.05).

Table 1. Effects of statin on serum concentrations of total cholesterol, triglyceride, HDL-C, LDL-C and the activity of lecithin:cholesterol acyl transferase in albino rats.

<table>
<thead>
<tr>
<th>Lipid Parameters</th>
<th>Group 1 (M ± S.D.)</th>
<th>Group 2 (M ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>1.84±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.98±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.86±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>1.33±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.25±.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>1.36±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL/CHDL-C</td>
<td>0.17±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free cholesterol (index of LCAT activity) (mg/dl)</td>
<td>0.06±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± S.D.; n=15. Values bearing different alphabets in the same row are significantly different (p<0.05). Group 1: animals given therapeutic dose of statin; Group 2: animals that were not given statin i.e. control.
HDL plays a prominent role in lipids metabolism. The inverse correlation between HDL levels and cardiovascular heart disease risk has been explained by the ability of HDL to remove cholesterol from the peripheral circulation and deliver it to the liver for excretion in the bile, in the process known as reverse cholesterol transport (Endo et al., 1977). Reduced serum concentration of high density lipoprotein, even in the presence of reduced low density lipoprotein, has been linked to the occurrence of cardiovascular disorder. Since the original seminal hypothesis of Gordon et al. was proposed in 1977 (Sposito and Chapman, 2002), the atheroprotective role associated with HDL has become widely recognized. A plethora of potential mechanisms that may account for the cardioprotective effects of HDL have been documented, several of which may be mutually interactive and, indeed, synergistic. ApoB100-containing particles deliver cholesterol to peripheral tissues and to the developing plaque, whereas HDL, primarily through the scavenger receptor class B type 1 (SR-BI/Cla-1) and LIMPII analogous 1 (CD-36) receptor on human macrophages, is able to pick up cholesterol from atherosclerotic plaque and return it to the liver for excretion in form of bile acids. Although reverse cholesterol transport is one of the major functions of HDL particles, HDL exerts several other potentially anti-atherogenic actions.

Simvastatin equally caused reduction in the mean serum concentrations of triglyceride, total cholesterol and LDL-C. In addition to decreasing LDL-C concentration through the inhibition of HMG-CoA reductase, the enzyme involved in the rate limiting step of cholesterol biosynthesis, statins lower triglycerides and modestly increase HDL-C (Matsuda et al., 1993). This has been further confirmed in this study as the mean value of HDL-C and mean value of TG was observed to be moderately increased and slightly decreased respectively. An overall decrease was observed in the mean concentration on total cholesterol, triglyceride and LDL-C of the test group. This overall decrease could be as a result of the lipid lowering effect of simvastatin which inhibited the enzyme HMG-CoA reductase involved in the rate limiting step of cholesterol biosynthesis. This finding is similar to the report of (Galle et al., 1994). Rosenfeld et al. (1983), reported that in the atherogenic dyslipidaemias, statins act to decrease levels of atherogenic lipoproteins and to re-establish equilibrium between cardioprotective HDL and atherogenic ApoB-containing lipoproteins. As a consequence, cholesterol efflux from the plaque is enhanced, whereas cholesterol influx from atherogenic lipoproteins is considerably diminished. A decrease in plaque cholesterol and macrophage content decreases inflammation and enhances plaque stability, resulting in a decrease in cardiovascular events. This is in line with the decreased mean concentrations of total cholesterol, LDL-C and triglyceride and increased mean concentration of HDL-C observed in this study.

HDL has various species, identified on the basis of their major apolipoprotein (apo) components (apoA-I or apoA-II), density (HDL2 and HDL3) and electrophoretic mobility (α and pre-β) (Vakeva et al., 1994). Changes in HDL levels more closely reflect variations in the HDL2 subfraction rather than HDL3. Several studies have shown that low levels of HDL2 and HDL3 are associated with increased progression of atherosclerosis and risk of cardiovascular disease (Fleisher et al., 1982). Since HDL and ApoA-I constitute major receptors of cholesterol in the cholesterol efflux, increasing HDL levels may increase cholesterol efflux and reverse cholesterol transport, contributing to reduced cardiovascular disease

### Table 2. Effect of the administration of simvastatin on body and visceral organs weights in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>body weight gain (g)</th>
<th>weight of liver (g)</th>
<th>weight of heart (g)</th>
<th>weight of kidney(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>45.13±3.05a</td>
<td>2.41±0.11a</td>
<td>0.38±0.02a</td>
<td>0.45±0.01a</td>
</tr>
<tr>
<td>Group 2(control)</td>
<td>60.20±1.64b</td>
<td>2.85±0.14a</td>
<td>0.41±0.07a</td>
<td>0.49±0.01a</td>
</tr>
</tbody>
</table>

Values are means ± SD; n=15. Values bearing different alphabets in the same column are significantly different (p<0.05). Group 1: animals given therapeutic dose of statin; Group 2: animals that were not given statin i.e. control.

### DISCUSSION

Hypercholesterolemia is a known and significant risk factors for the development of coronary heart disease (CHD) and death (Masunori et al., 2002). The epidemiological study established that a high concentration of serum cholesterol confers a high risk of CHD. Although, the use of statin in the management of hypercholesterolemia had been demonstrated as first-line pharmacotherapeutic agents, the effects of statin on atherosclerosis and lipid levels in animal models are very paradoxical (Guohua et al., 2011). While Bea et al. (1993; Assman et al., 2003 and Choudhury, 2002), showed that the administration of simvastatin to apoE-deficient mice caused a significant elevation of plasma cholesterol, Masunori et al. (2002) demonstrated that simvastatin caused a reduction in plasma concentration of total cholesterol. Our data was in agreement with Guohua et al., 2011 who reported that the administration of simvastatin to apoE-deficient mice fed a high cholesterol diet caused a reduction in plasma total cholesterol levels.

In this study, HDL-C concentration was increased by the administration of simvastatin. One of the objectives of this study was to determine and compare the concentrations of HDL-C in rats administered simvastatin and healthy controls and to investigate the relationship between possible changes in serum HDL-C concentration and the activity of lecithin:cholesterol acyl transferase. The administration of simvastatin significantly increased concentration of HDL-C. Although, previous studies reported the ability of statins to increase the concentration of HDL-C (Jones et al., 1998; Jones et al., 2003; Assman and Goto, 2004; Shabhangi et al., 2013), findings here further affirmed this capacity. A risk factor for the cardiovascular disease includes a low serum level of HDL-cholesterol. Low level of HDL-cholesterol has been linked to an increased risk of CVD through series of epidemiological and clinical studies (Barter et al., 2007). HDL-C is actively involved in reverse cholesterol transport; hence its low level plays a direct role in the atherogenic process. Raising HDL-C is widely encouraged as a means of reducing the predisposition to atherogenesis. In this study, treatment with simvastatin led to a significant elevation of plasma HDL-cholesterol, indicating its protective role against CVD. Studies have shown cardioprotective activities of statin administration (Gordon et al., 1977; Mackness et al., 2000).

Antihyperlipidemic and antiatherogenic activity of simvastatin was proposed in 1977 (Gordon et al., 2003; Assman et al., 1994). Changes in HDL levels more closely reflect variations in the HDL2 subfraction rather than HDL3. Several studies have shown that low levels of HDL2 and HDL3 are associated with increased progression of atherosclerosis and risk of cardiovascular disease (Fleisher et al., 1982). Since HDL and ApoA-I constitute major receptors of cholesterol in the cholesterol efflux, increasing HDL levels may increase cholesterol efflux and reverse cholesterol transport, contributing to reduced cardiovascular disease.
In this study, the body and organs weight in simvas-tatin administered rats were decreased significantly when compared with control animals. The hypolipidemic and antiatherogenic effects of simvastatin may be responsible for the beneficial action of this drug on body and organs weights. (Koller et al., 1982) reported similar findings with topiramate, atorvastatin per se and the combination of topi-mate + atorvastatin in cafeteria diet fed animals significantly decreasing the weight of kidney, liver, spleen and heart.

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