Effect of Atorvastatin on Serum Para-Oxonase-1 and C-Reactive Protein in Wistar Rats

Aradhana Marathe¹ · B. Ganaraja² · K. Ashwin Shenoy¹ · K. Ashok Prabhu¹ · M. Nandini¹

Received: 31 August 2017 / Accepted: 21 February 2018 / Published online: 17 March 2018
© Association of Clinical Biochemists of India 2018

Abstract Statins have been widely used in the treatment of hypercholesterolemia and atherosclerotic disease. Atherosclerosis is an ongoing inflammatory response which is involved in mediating all stages of this multifactorial disease. The present study focuses on the long term effect of atorvastatin on the anti-atherogenic and anti-inflammatory properties with reference to para-oxonase and C-reactive protein levels in rats. Thirty six Wistar albino rats obtained from the central animal house were divided into 6 groups with 6 rats in each group. Group I and IV served as the control for male and female rats respectively. Group II and V comprised of male and female rats that received low dose of atorvastatin (10 mg/kg body weight). Group III and VI comprised of male and female rats that received high dose of atorvastatin (40 mg/kg body weight) for period of 45 days. Blood was collected by cardiac puncture. The plasma was analysed for total cholesterol, HDL cholesterol, C-reactive protein (CRP) and Para-oxonase-1, both basal Para-oxonase (BPON) & Salt stimulated Para-oxonase (SPON) by standard procedures. Results of the present study showed a reduction in TC and increase in HDL-C in both groups of rats receiving low and high dose of Atorvastatin. Both male and female rats responded similarly. The levels of CRP decreased in the male rats receiving either low or high dose of atorvastatin. Activity

of SPON and BPON was increased only in the group receiving high dose of atorvastatin in both male and female rats.

Keywords Atorvastatin · Cholesterol · Para-oxonase-1 · Salt stimulated Para-oxonase (SPON) · Basal Para-oxonase (BPON) · CRP

Introduction

Statins have been widely used in the treatment of hypercholesterolemia and atherosclerotic disease. It is also reported that lipid lowering therapy with statins significantly decreases the cardiovascular mortality and morbidity. It is a well-known fact that Hypercholesterolemia is a major risk factor for atherogenesis. Its pathogenesis in turn involves an ongoing inflammatory response which is involved in mediating all stages of this disease from initiation, through progression and ultimately thrombotic complications of atherosclerosis. High levels of LDL contributes to the development of atherosclerosis whereas, HDL-cholesterol protects against it, thus, preventing the risk of heart attack and stroke [1]. Para-Oxonase, an antioxidant enzyme protects HDL from oxidation and is a major contributor to the anti-atherogenicity of this lipoprotein. Being an anti-oxidant enzyme, it breaks down the oxidized lipids and neutralizes its pro-inflammatory effects. Its serum concentration is influenced by inflammatory changes and the levels of oxidised LDL. There is considerable evidence that the antioxidant activity of high density lipoprotein (HDL) is largely due to the presence of Para-oxonase [2, 3]. Low grade chronic inflammation as indicated by the inflammatory marker C reactive protein (CRP), an acute phase reactant, defines the risk of

✉ M. Nandini
nandini.m@manipal.edu

¹ Department of Biochemistry, Kasturba Medical College, Mangaluru, Manipal Academy of Higher Education, Manipal, Karnataka, India

² Department of Physiology, Kasturba Medical College, Mangaluru, Manipal Academy of Higher Education, Manipal, Karnataka, India

atherosclerosis complication, thus adding to the prognostic information provided by the traditional risk factors [4].

Data from Heart protection study favours the prophylactic use of statins in patients with high risk of vascular events [5, 6]. Reportedly, statins exert their benefits through inhibition of de-novo cholesterol synthesis, resulting in significant reduction of total and LDL-cholesterol levels [7]. Despite early hypolipidemic effect by statins, at least 1 year of treatment was needed to detect significant changes in the plaque size. Simvastatin induced a significant reduction in total and LDL cholesterol at 6 weeks and had also induced vascular remodelling. No changes were seen at 6 months, whereas at 12 months, significant reduction in vascular wall thickness and vessel wall area without changes in lumen area were noted [8]. Reduction in plaque size and lipid core is seen in patients receiving an aggressive lipid altering regimen for 10 years. Statins which are widely used to treat hypercholesterolemia reduces the risk and also limits inflammation [9]. Although statins have been used in prophylactic treatment, the long term effects of statins are associated with potentially serious side effects such as myopathy as well as Diabetes Mellitus [10, 11].

Elevated levels of several inflammatory markers such as cytokines, cell adhesion molecules and acute phase reactants among apparently healthy men and women have proved to have predictive value for future vascular events. Studies have demonstrated that baseline CRP levels predict future cardiovascular events [9]. Both men and women with elevated levels of CRP consistently showed high vascular risk even in the absence of hyperlipidemia [12].

Studies on the effect of statins on para-oxonase is limited. The present study focusses on the long term effect of statins on the anti-atherogenic and anti-inflammatory properties in relation to para-oxonase in rats. The aim of the study was to compare the long term effects of prophylactic statin therapy (low dose and high dose) on para-oxonase and CRP at both baseline and after 45 days of treatment.

Materials and Methods

Thirty six albino rats (18 male rats and 18 female rats) were obtained from the Central Animal House after receiving the permission and clearance from Institutional animal ethics committee (IAEC). Animals were divided into six groups with 6 rats in each group. Sample size was based on previous studies which is sufficient to give a reliable result.

The division of the groups are as follows:

Group I (male rats) Control (placebo treated)

Group II (male rats)	Received low dose of statin (10 mg/kg body weight)
Group III (male rats)	Received high dose of statin (40 mg/kg body weight)
Group IV (female rats)	Control (placebo treated)
Group V (female rats)	Received low dose of statin (10 mg/kg body weight)
Group VI (female rats)	Received high dose of statin (40 mg/kg body weight)

The rats were housed in polypropylene cages with paddy husk bedding in standard laboratory conditions for 60 days. They were fed with normal pellet diet and water *ad libitum*. Two millilitres of blood was collected before treatment and at the end of study by cardiac puncture in ether anaesthesia. Atorvastatin (ATOCOR-10 mg) tablets were powdered and dissolved in 1 ml of water. Appropriate volume required according to the body weight was calculated and force-fed using gavage. After treatment blood was drawn to assess same parameters again. The whole blood thus collected before and after treatment was centrifuged for plasma separation. The plasma was analysed for the total cholesterol, HDL-cholesterol, and CRP by using AGAPPE DIAGNOSTICS kits in a semi auto analyser [13–15]. Para-oxonase activity, both basal and salt stimulated (BPON and SPON) was estimated by p-nitrophenylphosphate method [16].

Statistical Analysis

The pre and the post treatment values were compared by one way ANOVA followed by paired sample test. *p*-value of < 0.05 was considered as statistically significant.

Results

The body weights of the animals in the respective groups before and after treatment are presented in Fig. 1. An increase in the weight was seen after a period of 6 weeks in male rats only. There was no significant change in the body weight of the female rats. The values of total cholesterol obtained after statin therapy are presented in Table 1. Reduction in cholesterol seen in both low and high dose treated male as well as female rats is highly significant ($p < 0.001$). HDL-cholesterol levels observed after statin therapy are presented in Table 1. Increase in HDL-cholesterol after statin therapy is observed in both low and high dose treated male rats ($p = 0.002$ & $p \leq 0.001$) and female rats ($p = 0.003$ & $p \leq 0.001$) respectively.

Fig. 1 Depicts the body weight of the rats (g) with atorvastatin treatment

(n= the number of rats in each group)

Table 1 Effect of atorvastatin on total cholesterol (TC) and HDL cholesterol (HDL-C) levels in rats

n = 6	Pre-treatment (Mean $\pm$ SD) (mg/dl)	Post-treatment (Mean $\pm$ SD) (mg/dl)	p-value	Percentage change
Group 1				
TC	120.88 $\pm$ 2.44	120.53 $\pm$ 2.04	0.420	
HDL-C	35 $\pm$ 2.57	34.56 $\pm$ 0.9	0.817	
Group 2				
TC	120.88 $\pm$ 2.44	115.73 $\pm$ 2.78	0.001*	3.98%
HDL-C	35 $\pm$ 2.57	38.96 $\pm$ 3.23	0.002*	10.41%
Group 3				
TC	120.88 $\pm$ 2.44	107.83 $\pm$ 2.77	0.001*	10.53%
HDL-C	35 $\pm$ 2.57	42.9 $\pm$ 2.94	0.000*	24.13%
Group 4				
TC	121.61 $\pm$ 2.65	120.92 $\pm$ 1.68	0.340	
HDL-C	34.48 $\pm$ 3.04	34.77 $\pm$ 3.77	0.654	
Group 5				
TC	121.61 $\pm$ 2.65	116.68 $\pm$ 1.62	0.000*	3.50%
HDL-C	34.48 $\pm$ 3.04	39.01 $\pm$ 3.31	0.003*	12.19%
Group 6				
TC	121.61 $\pm$ 2.65	112.68 $\pm$ 1.62	0.001*	6.81%
HDL-C	34.48 $\pm$ 3.04	43.13 $\pm$ 3.2	0.000*	24.04%

n = the number of rats in each group, * = $p < 0.05$

TC and HDL-C levels in rats before and after the treatment

Treatment groups showed significantly reduced levels of TC and increased levels of HDL-C compared to the control (* $p < 0.05$)

The values of CRP obtained after statin therapy are presented in Table 2. Significant reduction in CRP is seen in both low and high dose treated male rats ($p = 0.036$

and 0.005 respectively), however the reduction seen in female rats was not statistically significant. The values of basal paraoxonase (BPON) obtained after statin therapy are

Table 2 Effect of Atorvastatin on CRP levels in rats

n = 6	Pre-treatment (Mean $\pm$ SD) (mg/L)	Post-treatment (Mean $\pm$ SD) (mg/L)	p-value	Percentage change
Group 1	0.346 $\pm$ 0.06	0.35 $\pm$ 0.08	0.465	
Group 2	0.346 $\pm$ 0.06	0.30 $\pm$ 0.06	0.036*	14.28%
Group 3	0.346 $\pm$ 0.06	0.28 $\pm$ 0.06	0.005*	20.00%
Group 4	0.276 $\pm$ 0.06	0.23 $\pm$ 0.05	0.086	
Group 5	0.276 $\pm$ 0.06	0.24 $\pm$ 0.06	0.081	4.16%
Group 6	0.276 $\pm$ 0.06	0.25 $\pm$ 0.04	0.355	8.00%

n = the number of rats in each group, * = $p < 0.05$

CRP levels in rats before and after the treatment

Treatment groups showed significantly reduced levels as compared to the control (* $p < 0.05$)

Table 3 Effect of Atorvastatin on Basal and Salt stimulated Paraoxonase (BPON & SPON) in rats

n = 6	Pre-treatment (Mean $\pm$ SD) (IU/L)	Post-treatment (Mean $\pm$ SD) (IU/L)	p-value	Percentage change
Group 1				
BPON	100.5 $\pm$ 12.84	101.87 $\pm$ 21.8	0.394	
SPON	68.75 $\pm$ 5.16	67.31 $\pm$ 4.54	0.118	
Group 2				
BPON	100.5 $\pm$ 12.84	81.03 $\pm$ 26.9	0.211	20.45%
SPON	68.75 $\pm$ 5.16	54.80 $\pm$ 10.76	0.062	18.58%
Group 3				
BPON	100.5 $\pm$ 12.84	127.58 $\pm$ 20.15	0.065	20.15%
SPON	68.75 $\pm$ 5.16	134.05 $\pm$ 46.72	0.021*	49.78%
Group 4				
BPON	76.68 $\pm$ 31.71	81.69 $\pm$ 45.73	0.077	
SPON	81.1 $\pm$ 34.65	79.13 $\pm$ 45.67	0.206	
Group 5				
BPON	76.68 $\pm$ 31.71	125.63 $\pm$ 32.76	0.004*	34.9%
SPON	81.1 $\pm$ 34.65	109.93 $\pm$ 72.01	0.288	28.00%
Group 6				
BPON	76.68 $\pm$ 31.71	127.54 $\pm$ 20.16	0.010*	35.9%
SPON	81.1 $\pm$ 34.65	134.04 $\pm$ 46.72	0.017*	40.96%

n = the number of rats in each group, * = $p < 0.05$

BPON and SPON levels in rats before and after the treatment

Treatment groups showed significant changes in male and female as compared to their controls (* $p < 0.05$)

presented in Table 3. Increased levels are observed in both the low and high dose treated groups of female rats only ($p = 0.004$ and $p = 0.01$ respectively). Low dose treated group of male rats showed a decrease while high dose treated group showed an increase although statistically not significant.

The values of Salt stimulated paraoxonase (SPON) obtained after statin therapy are presented in Table 3. Significant increase in SPON is observed only in high dose treated group of both male and female rats ($p = 0.021$ and 0.017). However the decrease observed in the low dose

treated male rats and the increase in the female rats is not statistically significant.

Discussion

Statins have been recognized as the most effective class of drugs for reducing the serum cholesterol levels and reduce the risk of cardiovascular morbidity and mortality. Atorvastatin, a synthetic reversible inhibitor of HMG-CoA reductase at a dose of 10 mg/kg body weight and 40 mg/kg

body weight was used to study the effect of statins on total cholesterol, HDL-cholesterol, CRP and para-oxonase activity (Both basal and salt stimulated) in normal rats for a period of 6 weeks.

The reduction in total cholesterol levels observed in male and female rats which received a lower dose Atorvastatin (Table 1) was similar (3.9 and 3.5% respectively). However, the reduction observed in the high dose treated groups was higher in the males than the female rats (10.53 and 6.8%). There was no significant change in the increase in HDL-cholesterol in male and female rats receiving either low or high dose of atorvastatin (10.41, 12.1, 24.1, 24.01% respectively). This finding is in agreement with the study of Adekunle and Bukoye [17]. The increase in HDL in the study is in contrast to the report of Masatoshi et al. [18] who reported that HDL levels remained unchanged after treatment with atorvastatin (5 mg/kg body weight) in humans. This could be attributed to the higher dosage of statins administered (40 mg/kg body weight) in our study.

Significant reduction in the CRP levels with atorvastatin is seen in both low and high dose treated groups of male rats only (Table 2) This is in agreement with the study of Masatoshi et al., who reported a decrease in hs-CRP levels only at 10 mg/kg body weight in humans. Statin induced hs-CRP levels have also been reported [19–21]. The observed reduction seen only in male rats in the present study could be attributed to the pleiotropic effect of atorvastatin. This is an interesting finding and needs to be delineated.

Para-oxonase is responsible for the anti-oxidant effect of HDL. Increase in both the basal (BPON) and salt stimulated (SPON) Paraoxonase-1 (PON-1) activity is seen in the groups treated with high dose of statins, indicating that statins exert their effect on PON-1. However, the male rats treated with the low dose showed a decrease in PON-1 activity, though not statistically significant (Table 3). This finding needs to be further analysed. However the increase in SPON activity observed in the high dose treated group was similar in male and female rats. Variation in the PON-1 activity in the healthy population exist and is attributed to the nutritional differences [22]. Changing from a standard laboratory chow to a cholesterol rich diet markedly decreases the serum PON-1 activity [23]. Degraded cooking oil has been reported to lower PON-1 in humans and alcohol has been reported to raise its activity [24, 25].

Some experimental evidences suggest that the decrease in PON-1 activity may occur as a part of an inflammatory response [26–28]. This has clinical implication since low PON-1 activity is associated with several diseases such as diabetic nephropathy, Parkinson's disease, organophosphate poisoning etc. The lipid lowering drug fibric acid and polyphenols have been reported to raise the PON-1 activity [29, 30]. This fact can be exploited for the use of statin

therapy in raising the PON-1 activity. Decreased PON-1 activity is associated with increased risk of CAD regardless of age [31]. In the present study, Atorvastatin treatment did not have any adverse effect on PON-1 activity.

Thus Atorvastatin, at low and high dose exhibited a beneficial effect on the normal rats as evidenced by the reduction in total cholesterol and increase in the HDL-cholesterol. Further it has increased the levels of both BPON and SPON activity along with the reduction with the CRP levels in male rats. The pleiotropic effect of Atorvastatin on CRP levels observed only in male rats needs to be further investigated. Increase in BPON activity in female rats receiving low dose of atorvastatin needs to be explored.

Conclusion

It may be concluded that appreciable lipid lowering effect by long term prophylactic treatment of Atorvastatin is beneficial. Elevation in the activity of PON-1 following prophylactic treatment with Atorvastatin is similar in both male and female rats. The findings of the present study suggests that the atorvastatin dose needed for the reduction of TC and CRP levels is not similar in male and female rats. Extrapolating this in humans may have clinical implications, suggesting that the outcome of statin therapy in males and females may not be the same and needs to be considered.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest.

References

1. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–43.
2. Ferretti G, Bacchetti T, Moroni C, Savino S, Liuzzi A, Balzola F. Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. *J Clin Endocrinol Metab*. 2005;90:1728–33.
3. Aviram M, Rosenbalt M, Biasgaier CH, Newton RS, Primo-Parmo SL. Paraoxonase inhibits high density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *Clin Invest*. 1998;101:1581–91.
4. Corti R, Fuster V, Fayad ZA, Worthley SG, Helft G, Smith D. Lipid lowering by simvastatin induces regression of human atherosclerotic lesions: two years follow-up by high-resolution noninvasive magnetic resonance imaging. *Circulation*. 2002;106:2884–7.
5. Durrington PN, Mackness B, Mckness MI. Paraoxonase and atherosclerosis. *Atheroscler Thromb Vasc Biol*. 2011;21:473–80.
6. Aronow WS. Hypercholesterolemia: the evidence supports use of statins. *Geriatrics*. 2003;58(8):18–20.

7. Aronow WS. Cholesterol: rationale for lipid-lowering in older patients with or without CAD. *Geriatrics*. 2001;56(9):22–5.
8. Delbosc S, Cristol J-P, Descamps B, Mimran A, Jover B. Hypertension, simvastatin prevents angiotensin II—induced cardiac alteration and oxidative stress. *Hypertension*. 2002;40:142–7.
9. Corti R, Fayad ZA, Fuster V, Worthley SG, Helft G, Smith D. Effects of lipid-lowering by simvastatin on human atherosclerotic lesions: a longitudinal study by high resolution, noninvasive magnetic resonance imaging. *Circulation*. 2001;104:249–52.
10. Bruckert E, Hayem G, Dejager S. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients—the PRIMO study. *Cardiovasc Drugs Ther*. 2005;19:403–14.
11. Sattar N, Preiss D, Murray H, Welsh P. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet*. 2010;375:735–42.
12. Ridker PM, Hennekens CH, Buring JE. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in Women. *N Engl J Med*. 2000;342:836–43.
13. Meitattini F, Prencipe L, Bardelli F, Giannini G, Tarli P. The 4-hydroxybenzoate/4-aminophenazone Chromogenic system used in the enzymatic determination of serum cholesterol. *Clin Chem*. 1978;4:2161–5.
14. Burstein M, Schonick HP, Morfin R. Cholesterol in high density lipoprotein using magnesium and phosphotungstic acid. *J Lipid Res*. 1978;19:583.
15. Rifal N, Tracy RP, Ridker PM. Clinical efficacy of an automated high sensitivity C-reactive protein. *Clin Chem*. 1999;45(12):2136–41.
16. Gan KN, Smolen A, Eckernon HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one ester catalyzing both activities. *Drug Metab Dispos*. 1991;19:100–6.
17. Adekunle AS, Bukoye OE. Assessment of anti-inflammatory activity of statin. *Int J Pharm Pharm Sci*. 2013;5(3):203–5.
18. Masatoshi F, Tatsuya M, Masaki I, Mayu T, Akiko I, Miwa K. Dose dependency in pleiotropic effects of atorvastatin. *Int J Anquiol*. 2007;16(3):89–91.
19. Rosenson RS, Koenig W. Utility of Inflammatory markers in the management of Coronary artery disease. *Am J Cardiol*. 2003;92:10i–8i.
20. Tekin A, Tekin G. Effects of atorvastatin on hemostatic and inflammatory parameters in hyperlipidemic patients with angiographically proven coronary artery disease. *Am J Cardiol*. 2004;94:206–9.
21. Aviram M, Sorenson R. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its Paraoxonase activities: selective active of human paraoxonase alloenzyme Q and R. *Arterioscler Thromb Vasc Biol*. 1998;10:1617–24.
22. Mackness MI, Mackness B, Hegele RA. Paraoxonase: biochemistry, genetics and relationship to plasma lipoprotein. *Curr Opin Lipidol*. 1996;7:69–76.
23. Mackness MI, Hennuyer M, Durrington PN. Paraoxonase activity is reduced by a pro atherosclerotic diet in rabbits. *Biochem Biophys Res Commun*. 2000;269:232–6.
24. Sutherland WHF, Walker RJ, de Jong SA, van Rij AM, Phillips V, Walker HL. Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. *Arterioscler Thromb Vasc Biol*. 1999;19:1340–7.
25. Van der Gaag MS, van Tol A, Scheek LM, James RW, Urgert R, Schaafsma G, et al. Daily moderate alcohol consumption increases serum paraoxonase activity: a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis*. 1999;147:405–10.
26. Van Lenten BJ, Hama SY, De Beer FC, Stafforini DM, McIntyre TM, Prescott SM, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. *J Clin Invest*. 1995;96:2758–67.
27. Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. *Atherosclerosis*. 1998;139:307–15.
28. Hedrick CC, Hassan K, Hough GP, Yoo J, Simzar S, Quinto CR. Short-term feeding of atherogenic diet to mice results in reduction of HDL and paraoxonase that may be mediated by an immune mechanism. *Arterioscler Thromb Vasc Biol*. 2000;20:1946–52.
29. Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs are potent antioxidant against lipoprotein oxidation. *Atherosclerosis*. 1998;138:271–80.
30. Paragh G, Balogh Z, Seres I, Harangi M, Boda J, Kovacs P. Effect of gemfibrozil on HDL-associated serum paraoxonase activity and lipoprotein profile in patients with hyperlipidaemia. *Clin Drug Invest*. 2000;19:277–82.
31. Zhao Y, Ma Y, Fang Y, Liu L, Wu S, Fu D. Association between PON1 activity and coronary heart disease risk: a meta-analysis based on 43 studies. *Mol Genet Metab*. 2012;105:141–8.