CLINICAL AND PRECLINICAL DATA ON COMBINATION THERAPY OF ATORVASTATIN AND AMLODIPINE: EFFECT ON LIPID PROFILE AND MUSCLE ACTIVITY

Mehjabeen*[1], Muhammad Arfat[2], Farah Owais[1], Noor Jahan[3], Asma Wazir[4], Sadaf Hina[1], Iffat Azeem[1], Hafiz Farrukh Niazi[1], Syeda Kiran[1]

[1]Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan
[2]Darson Institute of Science and Technology, Department of Pharmacy, Gujranwala and AIMS International University College, Pakistan
[3]Department of Pharmacology, Faculty of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[4]Department of Pharmacognosy, Faculty of Pharmacy, Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan

Abstract

Hypertensive patients with atherosclerosis are known to be vulnerable to the worsening of existing cardiovascular problems. This study was undertaken to assure, whether the combination therapy of calcium channel blocker,Amlodipine (AMD), and HMG CoA reductase inhibitor, Atorvastatin (ATV), is effective in reducing atherosclerosis possibly by lowering lipid profile. Three(3) groups of cardiovascular patients (n = 26), (both males and females; Age range 40 – 60 yrs) were selected. First and second groups were randomly given Amlodipine (10mg once daily), and Atorvastatin (40mg once daily) only, whereas combination of atorvastatin and amlodipine (Dose 10/40 once daily) was given to third group for 45 days. Plasma lipid profile of three groups was evaluated at Day 0, 15 and 45. For the purpose of pre-clinical studies, muscle relaxant and stimulant activity was evaluated on 20 mice (swiss Webster). Significantly lowered values ($P<0.0001$) of Total Cholestrol (TC), Very Low Density Lipoprotein (VLDL) and Triglycerides (TG) were observed with the adjuvant therapy of Amlodipine and Atorvastatin. Pre-clinical studies with mice gave sufficient evidences regarding the effect of atorvastatin on muscles which may lead to myopathy. Combination therapy of Amlodipine and Atorvastatin safely reduces LDL, Total Cholesterol (TC) and VLDL. It may have a beneficial effect on cardiovascular outcomes especially in coronary artery disease. Possible effect of atorvastatin on skeletal muscles, leading to muscle weakness followed by myopathy needs the dose optimization of this combination therapy.

Keywords. Atorvastatin, amlodipine, lipid profile, myopathy, atheroscelosis,

INTRODUCTION

Hypertension and Dyslipidemia are the most commonly co-occurring cardiovascular risk factors.Obesity is one of the significant factorthat causes dyslipidemia. Body-fat distribution could possibly be used to identify the subjects at the highest risk of distraught lipid profile and hypertension, as disturbed lipid profile has predominantly been associated with cardiovascular diseases. Coronary heart disease is more associated with the high level of LDL while high HDL gives the protective effect[1].
Atherosclerosis – isalso an important risk factor for heart disease. Atherosclerotic events can be avoided by lowering the lipids. It is either managed by diet control or by pharmacological therapy. Patients with obesity and having a metabolic syndrome, usually have typical dyslipidemia with reduced HDL-cholesterol (HDL-C) and sometimes hypertriglyceridaemia[2].

Atorvastatin – 3-hydroxy-3-methylglutaryl co enzyme A (HMG-CoA) reductase inhibitor – exerts direct anti-inflammatory, antioxidative, and vascular endothelial function-ameliorating pleiotropic effects. these effects are beyond lipid lowering effect, that could translate into a more significant prevention of cardiovascular disease [3].

Amlodipine – a calcium channel blocker – is usually prescribed in patients with angina pain along with other hypertensive patients. Combination therapy of Amlodipine and Atorvastatin holds advantage in treating hypertension and coronary artery disease effectively by treating complex dyslipidemias [4].

Atorvastain effectively reduce LDL and cholesterol concentration but myopathy – starting from myalgias to rare occurring rhabdomyolysis – being its side effect advocates its use with caution. Marcoff et.al. in 2007 suggest the hypothesis based on human data that coenzyme Q10 deficiency in skeletal muscle due to statin and mitochondrial dysfunction can be the cause of statin-associated myopathy but on low doses it does not affect coenzyme Q10. Their animal studies shows effect on ubiquinine concentration in skeletal muscles and mitochondrial dysfunction [5].

Materials and Methods

Clinical studies

We have evaluated retrospective data of 78 male and female patients – having age range of 40 to 60 years – treated with amlodipine, atorvastatin and their combination. The clinical data was obtained by the courtesy from Indus hospital and approved by institutional ethical committee of Indus hospital KarachiPakistan. Patients had normal diet and were not suffering from any infection. The present study focused on lipid profile only.

Dosing

Group I – Atorvastain40mg once daily
Group II – Amlodipine 10mgonce daily
Group III – Amlodipine + Atorvastatin 10&40 mg once daily respectively.

Biochemical analysis

Biochemical evaluations (lipid profile) were performed in patients at Day 0, 15 and 45 in the selected group of patients. Analyses of the total cholesterol, HDL, LDL, triglycerides and VLDL were performed in the clinical laboratory of Indus Hospital Karachi, as these are routine tests for the treatment and follow-up of the hypercholesterolimia. These determinations were performed using enzymatic, colorimetric methods. To evaluate these parameters, blood was collected from the patient in the mornings of Day 0, 15 and 45 of treatment after fasting state of 12hr.

Pre-clinical studies

20 mice,ranging from 30gm to 35 gm, were divided into 4 groups (n=5). One was control and rest of the groups were treated as mentioned in clinical studies above. Dose was adjusted according to the weight of mice. Different tests including traction test, rearing test, force swimming test and head dip tests were conducted in order to determine relaxant or stimulant activity.Observations were made after 30 minutes of oral administration of Amlodipine (0.15 mg/kg)andAtorvastatins(0.57 mg/kg) to the respective groups

Statistical analysis

Parameters obtained from lipid profile were compared by *t*-testand one way analysis of variance (ANOVA) with a repeated measures design between the pre- and post-treatment groups. P value greater than 0.05 was considered statistically significant [6].

RESULTS

Table 1 shows the changes in serum lipids profile after 45 day of treatment in group I. Serum concentrations of total cholesterol, LDL cholesterol, and triglycerides were significantly lowered ($p<0.05$), while HDL remain unchanged. The change in parameters of serum lipid profile in group II patients found to be non-significant. This effect was expected as amlodipine is a calcium channel blocker and doesn't possess any direct lipid lowering effect.A marked decrease in serum concentrations of total cholesterol, LDL cholesterol, and VLDL ($p<0.0001$), and triglycerides with ($p<0.05$) was found in group III. HDL cholesterol was least effected. However, changes started to appear from day 15 in total cholesterol and VLDL significantly.

In table 2, muscle relaxant and stimulant effect is shown by traction test, and forced swim test. Amlodipine in combination with Atorvastatin showed significant decrease in activity of muscles when compared with control.

Table 1: Effects of mono therapy of atorvastatin and amlodipine and their combination on lipid profile. Values are expressed in Mean ±SEM (n=26), P<0.05 – significantand P<0.0001 – highly significant.

		Day 0	Day 15	Day 45	P value Day 0 vs Day 15	P value Day 0 vs Day 45
Effect of Atorvastatin	Total cholesterol	281.17 ± 5.78	250.81 ± 5.10	190.26 ± 4.33	P<0.0003	P<0.0001
	Triglycerides	270.47 ± 9.47	232.24 ± 7.58	168.69 ± 5.96	P<0.0027	P<0.0001
	HDL	46.18 ± 0.61	45.37 ± 0.97	45.88 ± 0.72	P<0.4796	P<0.7527
	LDL	162.20 ± 3.90	140.91 ± 3.87	99.91 ± 3.12	P<0.0003	P<0.0001
	VLDL	41.31 ± 2.02	45.19 ± 1.35	32.94 ± 1.13	P<0.1167	P<0.0007
Effect of Amlodipine	Total cholesterol	262 ± 5.93	254 ± 6.35	263 ± 5.04	P<0.0900	P<0.1789
	Triglycerides	157 ± 4.81	150 ± 7.22	158 ± 4.98	P<0.1518	P<1.000
	HDL	47 ± 6.06	42 ± 3.76	50 ± 2.73	P<0.1033	P<0.6107
	LDL	134 ± 9.26	141 ± 6.64	120 ± 6.57	P<0.2101	P<0.1755
	VLDL	35 ± 1.76	52 ± 6.39	48 ± 6.11	P<0.0863	P<0.0499
Effects of Atorvastatin and amlodipine in combination	Total cholesterol	299.89 ± 7.63	265.27 ± 3.04	239.13 ± 2.18	P<0.0001	P<0.0001
	Triglycerides	273.28 ± 9.31	244.76 ± 6.80	225.85 ± 7.01	P<0.0168	P<0.0002
	HDL	48.03 ± 1.53	46.06 ± 0.97	45.34 ± 0.92	P<0.2809	P<0.1373
	LDL	182.77 ± 5.82	167.69 ± 3.73	149.81 ± 3.05	P<0.0339	P<0.0001
	VLDL	53.86 ± 2.04	44.35 ± 1.13	42.46 ± 1.28	P<0.0002	P<0.0001

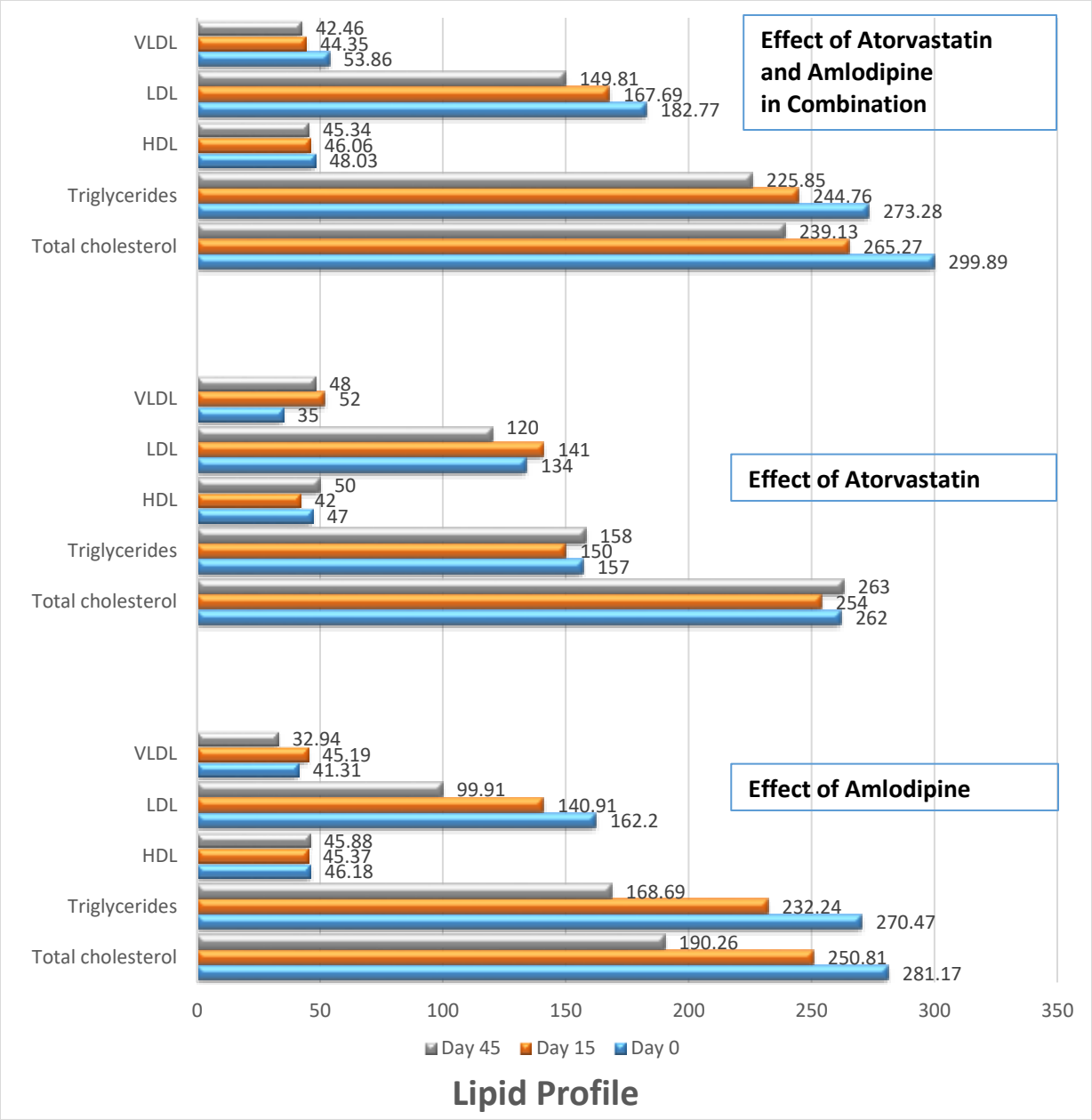

Figure 1: Effects of atorvastatin and amlodipine and in combination on lipid profile of patients on Day 0, 15 & 45

Table 2: Neuropharmacologicaltests : Pre-clinical testing for Atorvastatin and Amlodipine Values are expressed in Mean±SEM, n=5, p<0.05 – significant; p<0.0001 – highly significant

		Open Field	Rearing	Traction test	Head Dip	Cage Cross	Forced Swim Test
Effect of Atorvastatin							
	Control	224.50 ± 48.48	44.17 ± 8.70	10.17 ± 1.25	14.00 ± 1.03	53.17 ± 4.81	3.75 ± 0.17
	Treated	202.33 ± 42.06	21.50 ± 2.75	8.67 ± 0.84	7.00 ± 1.48	39.00 ± 1.37	3.58 ± 0.18
	***P*value**	P < 0.7370	P < 0.0324	P < 0.3432	P < 0.0031	P < 0.0177	P < 0.5022
Effect of Amlodipine							
	Control	85.83 ± 8.86	16.67 ± 1.84	11.17 ± 0.91	11.50 ± 2.29	35.15 ± 2.12	2.87 ± 0.60
	Treated	98.50 ± 8.84	11.50 ± 1.71	12.67 ± 1.02	14.00 ± 2.05	34.67 ± 2.23	3.64 ± 0.19
	***P* value**	P < 0.3260	P < 0.0665	P < 0.2987	P < 0.4350	P < 0.8742	P < 0.1093
Effects of Atorvastatin and Amlodipine in combination							
	Control	85 ± 8.86	16.67 ± 1.84	11.17 ± 0.91	11.50 ± 2.29	35.15 ± 2.12	2.87 ± 0.60
	Treated	80 ± 3.84	14 ± 1.15	10 ± 0.72	12.00 ± 1.05	32 ± 1.23	2.10 ± 0.30
	***P* Value**	P < 0.4280	P < 0.0761	P < 0.2879	P < 0.9310	P < 0.8042	P < 0.1034

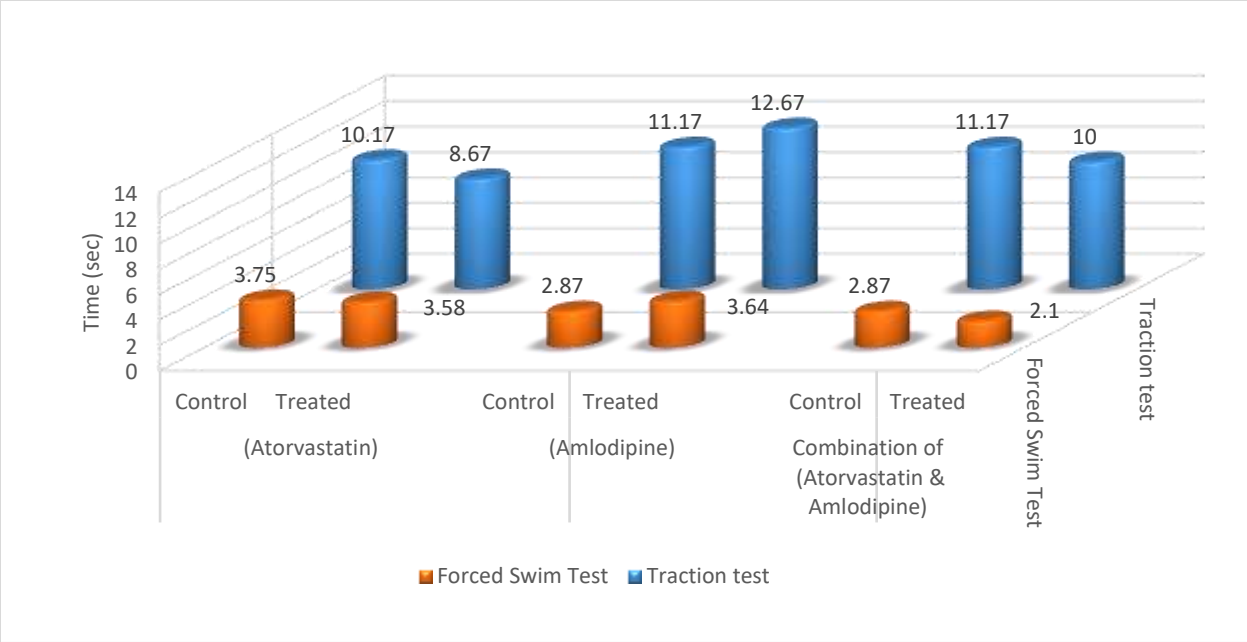

Figure 2: Effect of Atorvastatin and Amlodipine on skeletal muscle

Discussion

The lipid lowering effect is more progressive in combination of drugs. It may be due to its chemical structure and its molecular interaction with phospholipids of the cellular structure [4].

The synergistic anti-oxidant effect of amlodipine and atorvastatin causes lipid peroxidation in human low density lipoproteins and free fatty acids. The combination of this drug is use to treat cardiovascular disease or coronary artery disease by showing resistance to LDL[7].

Amlodipine has no favourableeffect on the lipid profile[8]. Atorvastatin, beinga HMG-Co reductaseinhibitor, - which is precursor of mevalonate in biosynthesis pathway of cholesterol – showsits potential effect on lowering TC, LDL and triglycerides.Combination of both drugs showeda potential effect in lowering TC, LDL and VLDL[9].

Table – 1 indicates a synergistic effect of amlodipine and atorvastatin on lipid lowering effect. It can possibly be explained by the increased accumulation of atorvastatin induced by amlodipine, atorvastatin are metabolized by the CYP3A4 isoenzyme system[10]. The calcium antagonist amlodipine is also metabolized by CYP3A4. The co administration of aorvastatin and amlodipine may increase the peak concentration (C_{max}) of HMG-Co reductase inhibitor and area under the concentration-time curve may also increased. The pharmacodynemic and pharmacokinetic study of amlodipine and simvastatin was performed [11].

It shows effective simultaneous treatment of dyslipidemia and hypertension or cardiovascular disorder. But this accumulation of statin also leads to myopathy as side effect in chronic use of atorvastatin [12]. Our preclinical studies showed a significant effect on muscles (Table – 2). In

open field there was a slight decrease in no. of movements for atorvastatin treated group. Traction test in which the griping of mice is effected due to relaxant effect of muscles. Forced swimming test also shows relaxant activity of muscles by decreasing the time for swim. These decreasing patterns of movement associated with atorvastatin which can lead myopathy.

The possible mechanism of muscle relaxant activity could be the decreased effect on mitochondrial function. Through electron transport chain mechanism number of ATPs are produced and for proper functioning of ETC ubiquinone or CoQ_{10} is required and mevalonate is responsible for its synthesis. The HMG-Co reductase inhibition property of statin also alter the synthesis of mevalonate which leads to myopathy as an adverse effect [13].

REFRENCES

1. Després, J-P, I Lemieux, G-R Dagenais, B Cantin, and Bt Lamarche, HDL-cholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. Atherosclerosis 2000;153:2:263-272.
2. Al-Ajlan, AR, Lipid profile in relation to anthropometric measurements among college male students in Riyadh, Saudi Arabia: A cross-sectional study. Int. J. Biomed. Sci. 2011;7:2:112-119.
3. Bellosta, S, N Ferri, L Arnaboldi, and F Bernini, Pleiotropic effects of statins in atherosclerosis and diabetes. Diabetes Care 2000;23:B72.
4. Mason, RP, *Synergistic effects of amlodipine and atorvastatin metabolite as a basis for combination therapy*, 2006, Google Patents.
5. Marcoff, L and PD Thompson, The role of coenzyme Q10 in statin-associated myopathy: a systematic review. J. Am. Coll. Cardiol. 2007;49:23:2231-2237.
6. Dar, UK, F Owais, M Ahmad, and GH Rizwani, Biochemical analysis of the crude extract of Momordica charantia (L.). Pak. J. Pharm. Sci. 2014;27:6 Spec No.:2237-2240.
7. Ge, C-J, S-Z Lu, Y-D Chen, X-F Wu, S-J Hu, and Y Ji, Synergistic effect of amlodipine and atorvastatin on blood pressure, left ventricular remodeling, and C-reactive protein in hypertensive patients with primary hypercholesterolemia. Heart Vessels 2008;23:2:91-95.
8. Osterloh, I, The safety of amlodipine. Am. Heart J. 1989;118:5:1114-1120.
9. Thompson, PD, P Clarkson, and RH Karas, Statin-associated myopathy. JAMA 2003;289:13:1681-1690.
10. Harper, CR and TA Jacobson, Avoiding statin myopathy: understanding key drug interactions. Clin. Lipidol. 2011;6:6:665-674.
11. Nishio, S, H Watanabe, K Kosuge, S Uchida, H Hayashi, and K Ohashi, Interaction between amlodipine and simvastatin in patients with hypercholesterolemia and hypertension. Hypertens. Res. 2005;28:3:223-227.

12. Evans, M and A Rees, Effects of HMG-CoA reductase inhibitors on skeletal muscle. Drug Saf. 2002;25:9:649-663.

13. Rundek, T, A Naini, R Sacco, K Coates, and S DiMauro, Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. Arch. Neurol. 2004;61:6:889-892.