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Effect of Atorvastatin Calcium on Nitric Oxide Concentration in Plasma of Rats

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ABSTRACT

Atorvastatin Calcium is a synthetic lipid-lowering agent and that belongs to the drug class known as Statins. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase Nitric oxide (NO) is an important chemical messenger of many physiological and biochemical processes, including thrombosis, inflammation, immunity, vasodilation, and neurotransmission. Hyperlipidemia is a disorder of lipoprotein metabolism, which allows a number of abnormalities such as hypercholesterolemia and hypertriglyceridemia. In recent times the World Health Organization (WHO) has confirmed that by 2020, 60% of cardiovascular cases will be of Indian origin. Statins are the drug of choice in the treatment for lowering lipid levels. Statins have to turn into the leading prescription drug. Indications of statins have been really absolute over the last 5 years successive to the publication of many multicenter prospective trials. Nitric oxide can be easily detected by treating the serum with Griess reagent. Nitric oxide concentrations were determined once in a week for a period of four weeks and observed for change in the concentration. The linear regression equation was Optical Density (O.D)=0.0003 × Concentration-0.0019, (r^2=0.999). These results were compared with blank plasma samples withdrawn from rats. The increased concentration was observed in the treatment group and maintained for the remaining weeks. This ensured that NO release in the blood with the administration of atorvastatin calcium is deemed to be involved in vasodilation of blood vessels and preventing the accumulation of atherosclerotic plaques and helpful maintenance of circulation.

Keywords: Atorvastatin, Nitric oxide, HMGA-CoA reductase, Neurotransmission, Griess reagent.

Abbreviations:LDL-C: Low-Density Lipoprotein-Cholesterol; EDRF: Endothelium-Derived Relaxing Factor; eNOS: endothelium Nitric Oxide Synthase; HDL-C: High-Density Lipoprotein Cholesterol; OD: Optical Density

INTRODUCTION

Atorvastatin Calcium is a synthetic lipid-lowering agent and belonging to the drug class known as Statins. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase is an enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis [1,2]. The main advantages of HMG-COA reductase inhibitors are decreases mortality due to antioxidant properties and eNOS expression activities [3]. Statins involved in the down regulation of eNOS expression induced by hypoxia and improve the vascular activity of NO and useful for the treatment of coronary heart disease [4]. Nitric oxide is a

strong vasodilator with a half-life of a few seconds in the blood and important in protecting the liver from ischemic failure. Nitric oxide is also known as the 'Endothelium-Derived Relaxing Factor', or 'EDRF', is produced from L-arginine and nitric oxide synthase. Nitric oxide, also known as nitrogen monoxide, is a molecule with chemical formula NO. Nitric oxide relaxes the vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cGMP lead to vasodilation [5,6]. Atorvastatin Calcium is used for Atherosclerotic vascular disease due to Hypercholesterolemia and mixed dyslipidemia, homozygous familial primary dysbetalipoproteinemia and/or "hypertriglyceridemia" as an adjunct to dietary therapy to decrease serum total and Low-Lipoprotein Cholesterol (LDL-C), "Apolipoprotein B"(apoB) and triglyceride concentrations, while increasing High Density Lipoprotein Cholesterol (HDL-C) levels [7,8].

Hyperlipidemia is a disorder of lipoprotein metabolism, which allows a number of abnormalities such as hypercholesterolemia and hypertriglyceridemia [9]. In recent times the World Health Organization (WHO) has confirmed that by 2020, 60% of cardiovascular cases will be of Indian origin [10]. Statins are the drug of choice in the treatment for lowering lipid levels. management of "hyperlipidemia" with statins has become an important part of the management of vascular diseases [11]. Statins have to turn into the leading prescription drug. Indications of statins have been really absolute over the last 5 years successive to the publication of many multicenter prospective trials. There are multiple statins available in the Indian market like atorvastatin, simvastatin, pravastatin, pitavastatin, fluvastatin, cerivastatin and rosuvastatin [12]. Most of the trials compared the fixed-dose regimens of more intensive statin therapy with less intensive statin therapy [13]. Van Dam et al. concluded that Atorvastatin proved to be both effective and safe when given as monotherapy in the treatment of severe combined dyslipidemia and familial dysbetalipoproteinemia [14]. The human cutaneous circulation is an accessible, representative regional circulation for investigating mechanisms of microvascular dysfunction [1,5,13]. Microvascular dysfunction occurring in the cutaneous circulation parallels changes that occur in the conduit arteries [7] and renal vascular beds [4]. We have recently demonstrated that the reduction in cutaneous NO-dependent vasodilation with hypercholesterolemia is, in part, mediated by an increase in arginase activity [15,16]. Increased arginase activity reduces substrate availability for "NO synthase" (NOS) and induces uncoupling of the enzyme into its monomeric form [17-19] resulting in decreased functional NO synthesis and increased oxidant stress through augmented superoxide production [20-22]. Furthermore, 3 mo of atorvastatin intervention decreases arginase activity, possibly through one of the pleiotropic effects i.e., the overall benefits observed with statins appear to be greater than what might be expected from changes in lipid concentrations alone of the statin (3-hydroxy-3-methyl-glutaryl-CoA reductase-inhibitors). Atorvastatin Calcium structure in Figure 1.

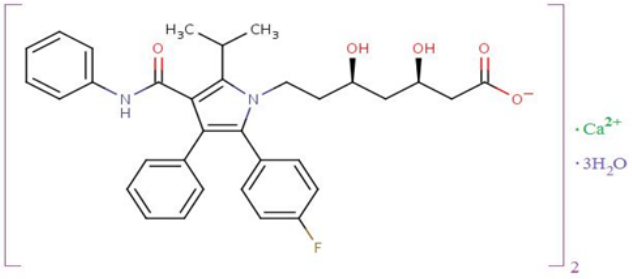

Figure 1: Atorvastatin calcium.

- Molecular formula: $(C_{34}\ H_{34}\ FN_{205})_2Ca.3H_2O$
- Molecular Weight: 1209.42
- IUPAC Name: [R-(R^*R^*)]-2-(4-fluoropheny1)-β, δ-dihydroxy-5-(1-methylethyl)-3-pheny1-4-[(phenylamino) carbony1]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

MATERIALS AND METHODS

Preparation of reagents

Accurately weighed 100 mg N-(1-naphthyl) ethylenediamine dihydrochloride was dissolved in 100 ml of distilled water. Accurately weighed 1 g of sulfanilic acid was dissolved in phosphoric acid solution, 5% v/v. Standard sodium nitrite was prepared by dissolving 10 mg in 100 ml of distilled water.

Calibration curve for standard sodium nitrite

The series of different concentrations such as 100, 200, 300, 500, 1000, 1500, 2000, 3000, 3500 ng/ml were prepared and treated with equal volumes of premixed N(1-naphthyl) ethylenediamine dihydrochloride and sulfanilic acid reagents (Griess reagent) and respective optical densities were measured at 548 nm by double beam UV-Visible Spectrophotometer Lab India (UV 3092), connected to digital system loaded with UV win software 5.2.0.1104 having a wavelength accuracy of L 5.0 with quartz cells of lcm path length (Table 1) and (Figures 2 and 3).

Table 1: Calibration data for NO determination by Griess Reagent Method.

S.No.	Conc (ng/ml)	Average O.D.	Std. Dev Δ	R.S.D §
1	100	0.029	0.0005	1.72
2	200	0.059	0.0006	1.01
3	300	0.084	0.0005	0.59
4	500	0.145	0.0008	0.55
5	1000	0.304	0.0025	0.82
6	1500	0.415	0.0025	0.6
7	2000	0.563	0.0051	0.9
8	3000	0.871	0.0048	0.55
9	3500	1.026	0.0031	0.3
Δ=Standard Deviation §=Relative Standard Deviation				

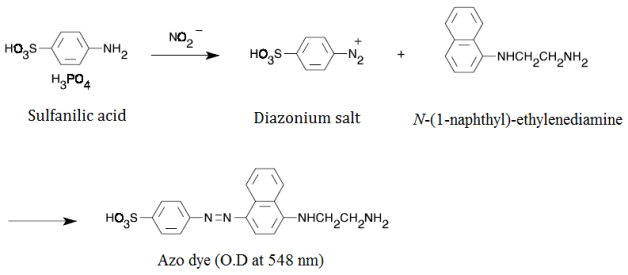

Figure 2: Formation of azo dye.

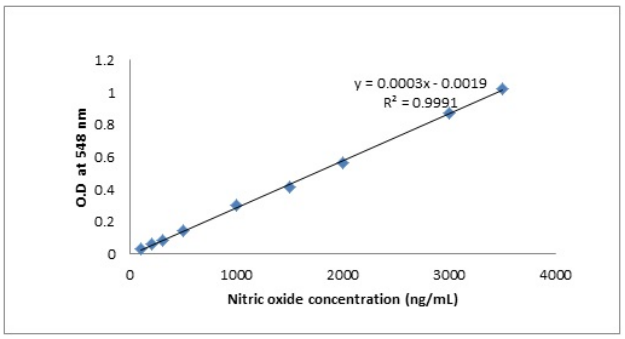

Figure 3: Calibration curve for Nitric oxide.

Determination of plasma nitric oxide

Blood was collected in heparinized 2 mL Thermo centrifuge tubes and allowed to centrifugation at 4000 rpm for 10 min at 4°C and plasma was separated and deproteinized with 15 mg/mL of zinc sulfate solution, then 0.5 mL of

supernatant was added to 0.5 mL of Griess reagent. After 10 min of color development at room temperature, the absorbance was measured at 548 mu and compared to a sodium nitrite calibration curve (Figure 4).

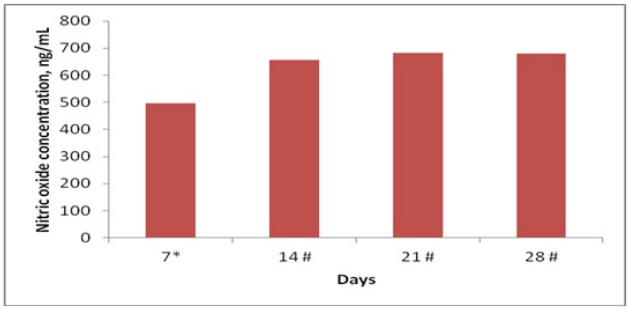

Figure 4: Plasma concentration of NO where * represents Control plasma conc and # represents Treatment group.

Studies in rats

Healthy Wistar albino rats of four groups (each group contains n=6 rats) either sex weighing 200-240 g were procured from the central animal house of the Balaji Institute of Pharmaceutical Sciences. The animals were housed in polypropylene cages and allowed to acclimatize for 7 days. All the animals were maintained under standard husbandry conditions of 12:12 h light: dark cycle at a temperature of 25°C with free access to standard rat pellet diet and water.

The experimental protocol was approved by the Institutional Animal Ethics Committee of "Balaji Institute of Pharmaceutical Sciences" Warangal, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (registration no. 1694/po/RE/S/2013/CPCSEA).

Administration of atorvastatin calcium in rats

Atorvastatin calcium was taken in polyethyleneglycol 400, 25% (v/v) administered orally by using standard gauge bloated tip needle every day for a period of four weeks. The control and treatment group was maintained throughout the study. Blood samples once in a week were withdrawn from tails of rats under mild ether anesthesia.

RESULTS AND DISCUSSIONS

The linear regression equation was Optical Density (0.D)=0.0003 × Concentration-0.0019, (/=0.9991) were shown in Figure 3. Nitric oxide concentrations were determined once in a week for a period of four weeks and observed for change in the concentration (Table 2). These results were compared with blank plasma samples withdrawn from rats. The increased concentration was observed in the treatment group and maintained for the remaining weeks. This ensured that NO release in the blood with the administration of Atorvastatin Calcium (ATC) is deemed to be involved in vasodilation of blood vessels and preventing the accumulation of atherosclerotic plaques and helpful maintenance of circulation.

Table 2: Plasma concentration of NO.

No. of days	NO conc in ng/mL ± Std. Dev Δ	R.S.D §
7	496.08 ± 15.44	3.11
14	656.33 ± 13.51	2.05
21	683.15 ± 14.86	2.17
28	679.67 ± 16.29	2.39
Δ=Standard Deviation §=Relative Standard Deviation		

O. Saluveer et al. [23] explains that a single oral dose of atorvastatin affects peripheral vascular reactivity in hypertensive subjects. Basal blood flow increased while the EDV was unaffected by atorvastatin. Ang II effect on vasoconstriction was inhibited by atorvastatin. The results suggest acute statin effects in hypertension that are

independent of the endothelium, indicating that VSMCs are affected more rapidly than the endothelial cells. These actions may in part contribute to the beneficial pleiotropic effects of statins.

G. Heeba et al. [24] explains that Atherosclerosis induced an endothelial [NO]/[ONOO−] balance indicative of endothelial dysfunction. Statins showed anti-atherosclerotic effects mediated by HO-1/eNOS, restoring the [NO]/[ONOO−] imbalance and reducing lipid peroxidation.

CONCLUSION

These results were compared with blank plasma samples withdrawn from rats. The increased concentration was observed in the treatment group and maintained for the remaining weeks.

This ensured that, NO release in the blood with the administration of atorvastatin calcium is deemed to be involved in vasodilation of blood vessels and preventing the accumulation of atherosclerotic plaques and helpful maintenance of circulation.

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