

The Possible Link Between Atorvastatin and Cataract in Rats: An Experimental Study

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Abstract: Atorvastatin is a member of drugs known as statins that responsible for lowering lipids to prevent cardiovascular disease. The purpose of this study is to determine potential cataractogenic action of atorvastatin in rats. Almost 75 healthy albino Wistar rats were classified into four groups: Group 1 served as control, Group 2 were animals received hypercholesterolemic diet, Group 3 and 4 that animals received hypercholesterolemic diet with atorvastatin in two doses 1.14 and 11.4 mg/kg/day, respectively. Activity of Na⁺-K⁺ ATPase was measured in lens membrane. Soluble lens proteins and Refractive Index (RI) were detected. In addition Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was done for all the studied groups. A significant decrease ($p < 0.05$) were observed in activity of Na⁺-K⁺ ATPase in lens membrane and soluble lens proteins of group 4 that received a high dose of atorvastatin in contrast to all others groups. There were remarkable shifts in molecular weight of all protein fractions detected in pattern of SDS-PAGE come with atorvastatin administrated (Group 4). Also, the same phenomena in appearing no variation in its value of RI to Group 2 or 3 and a significant difference (increase, $p < 0.05$) observed in Group 4. Lens turned out to be reacted to atorvastatin, especially, high doses administrated.

Key words: Atorvastatin, cataract, rat, lens, proteins, HDL

INTRODUCTION

Cataract is described as opacity in the crystalline of lens that prevents or disrupts the light that enters the eye. Pascolini and Mariotti (2012) found that cataract is the leading cause of preventable blindness and it is directly linked to 51% of cases. A surgery of expelling the cloudy shady natural lens and replacing it with an artificial, transparent intraocular lens was detected to be the only solution (Prajna *et al.*, 2015). All cataract surgeries are subject to complications post-surgery. Constant infection and cystoid macular edema can cause postoperative vision loss. Infectious endophthalmitis whereas rare is another serious vision-threatening complication (Tyson *et al.*, 2017).

Many medications and different xenobiotics may achieve systemic concentrations where they communicate not just with the proteins that are their helpful targets alter the cell membrane physicochemical properties which may modify capacity of numerous transmembrane proteins past the planned targets. These progressions in bilayer properties may add to nonspecific changes in membrane protein and cell work, since, layer proteins are energetic coupled to their host lipid bilayer (Redondo-Morata *et al.*, 2016).

A set of medications called 3-Hydroxyl-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors or “statins” included atorvastatin. It decreases the levels of “bad” cholesterol (Low-Density Lipoprotein or LDL) and triglycerides in the blood whilst increasing levels of “good” cholesterol (High-Density Lipoprotein or HDL) (Izadpanah *et al.*, 2015). Atorvastatin is the remedy for high cholesterol and to lower the danger of heart attack, stroke or other heart complications in type 2 diabetes patients, coronary heart disease or other risk factors.

Statin medicines work by obstructing the function of the liver enzyme that is the lead driver for producing cholesterol results in reduction of plasma cholesterol, causing increased synthesis of hepatic cell surface low-LDL receptors. This allows an improved hepatic absorbance of plasma LDL with reduced flowing levels (Pascolini and Mariotti, 2012).

Not only they are greatly clinically beneficial but also the long-term of taken statins has been directly linked with unfavorable effects including myopathy and neurological side effects (Hamann *et al.*, 2013; Murinson *et al.*, 2012; Tierney *et al.*, 2013). Statin therapy is linked to a heightened risk of diabetes (Wang *et al.*, 2017). Sattar *et al.* (2010) suggested that

clinical practice on patients with direct or high cardiovascular hazard or existing cardiovascular disease shouldn't change, since, the hazard is low both in absolute terms and once compared with the reduction in coronary events.

Zakrzewski *et al.* (2002) focus on the potential cataractogenic input of atorvastatin and observed by the optic microscope, the changes in the examined rat lens design could be a resulting influence of atorvastatin effect. Hippisley-Cox and Coupland (2010) found that statin was directly linked with an increased hazard of cataract in both males and females and no evident dose-response relation was found. Casula *et al.* (2016) analyzed the relation between exposure to statins and hospitalization for cataract and concluded that the therapy was related to a small rise in hazard of cataract surgery.

In the current studies, the link of atorvastatin treatment with cataract using different concentration and induced hypercholesterol case in rats were investigated in a point of view in the protein lens study.

MATERIALS AND METHODS

Chemicals supplies: All chemicals used in the experiments were provided by Sigma Company (St. Louis, MO, USA) with the most superior level of purity grad available.

Animals: About 75 healthy both male and female albino Wistar rats weighing 150-200 g (10 weeks) were the subjects for this study. They were housed in extraordinary outlined confines and kept up under consistent wind stream and brightening amid the exploratory periods, the rats were bolstered with adjusted eating routine. They were housed in distinct designed cages and kept up under constant air flow and lighting during the investigational periods, the rats were nourished with adjusted eating routine (Protein 21, starch 70, fat 3.5, fiber 3.5, minerals and vitamins 2%) and drink water ad libitum. The association for research in vision and ophthalmology statements and regulations were accordingly applied on the animals in the study. Normal animals (n = 6) denoted by group 1. Rest of animals take cholesterol extra pure powder made in to 20% suspension in coconut oil. The suspension was administered at the dose of 5 mL/kg/day at night hours for 30 continuous days (Ramachandran *et al.*, 2011).

Blood samples were preformed on all rats after 12 h fast. Serum was got by centrifugation of blood at 3,000 rpm for 20 min. Serum was analyzed further for assay of triglyceride, total cholesterol and high-density lipoprotein cholesterol according to the usual protocols given along

with respective kits (HDL and LDL/VLDL cholesterol assay kit (ab65390)). Animals having significant increase in serum triglyceride, total cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol level when compared with normal animals were considered as hypercholesterolemic while others which did not satisfy this conditions were excluded from the experiment. Hypercholesterolemic rats were categorized into the following groups:

- Group 2: hypercholesterolemic animals received cholesterol in oil (5 mL/kg/day)
- Group 3: hypercholesterolemic animals received atorvastatin (1.14 mg/kg/day)
- Group 4: hypercholesterolemic animals received atorvastatin (11.4 mg/kg/day)

Measurement of Na⁺-K⁺ ATPase activity on the lens membrane: All groups of animals were decapitated after 4 weeks and eyes were enucleate from the eye globe, then the lenses were freed from the eye and their capsules were carefully removed. Lens capsules were weighed in separate vessels then homogenized in extraction medium (0.32 M sucrose, 1 M EDTA and 0.15% deoxycholic acid). Na⁺-K⁺ ATPase measurement was practiced on the membrane of lens by Bowler and Tirri (1974) method.

Measurement of total soluble lens proteins: The lenses were weighed without their capsules and then homogenized separately in de-ionized water. They were then centrifuged at 16,000 RPM to extract soluble lens proteins. Total amount of proteins in the soluble part of the lens crystalline were pinpointed by Lowry *et al.* (1951) method.

SDS polyacrylamide gel electrophoresis: Depending on their molecular weights soluble proteins were separated by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to Laemmli (1970) by 5% stacking gel and 12% separating gel. The resultant data were shown graphically with an automatic scanner (Model R-112, manufactured by Beckman).

Measurements of the refractive index: The refractive index was measured using Zeiss Abbe refractometer attached to temperature control unit type mg W Lauda made in Germany. The temperature was fixated at 25±10°C during measurements. Abbe's refractometer is used as a specific monochromatic light source the apparatus was adjusted with water as the liquid.

Statistical analysis: Mean standard deviations were expressed through statistical analysis. Comparisons

among multiple groups were made by using Analysis of Variance (ANOVA). Commercially available Statistical Software Package (SPSS-11 for Windows) was used and the significance level was established at $p < 0.05$.

RESULTS AND DISCUSSION

Figure 1 is a histogram indicated the changes of measurements of $\text{Na}^+\text{-K}^+$ ATPase activity in imoles of Pi liberated/min/mg protein to hypercholesterolemic animals (Group 2) and hypercholesterolemic animals treated with 1.14 mg/kg/day of atorvastatin (Group 3) and 11.4 mg/kg/day of atorvastatin (group 4). Neither Group 2 nor Group 3 had evident variances in value of $\text{Na}^+\text{-K}^+$ ATPase activity compared to control that had the value 13.34 ± 0.4 μmoles of Pi liberated/min/mg protein. After treated the hypercholesterolemic animals with 11.4 mg/kg/day of atorvastatin (Group 4), there was a significant decrease ($p < 0.05$) in $\text{Na}^+\text{-K}^+$ ATPase activity of lens membrane and reached to 9.2 ± 0.6 μmoles of Pi liberated/min/mg protein.

Figure 2 indicated the total soluble lens proteins for all the studied groups. The control value was 275.9 ± 6 mg/g wet wt. Also, no apparent difference in total soluble proteins in Group 2 or 3 but a significant decrease ($p < 0.05$) was observed for Group 4 that treated with highly dose of atorvastatin.

Figure 3 indicated the electrophoretic pattern for control (Group 1), hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+11.4 mg/kg/day of atorvastatin (Group 4). The control panel revealed eight peaks varies in their width, intensity and cover the molecular weight range $177 \pm 4\text{-}12 \pm 1$ kDa. Group 2 and 3 revealed no change in the number of peaks or the molecular weight of the proteins peaks. But Group 4 suffered from shift to high molecular, decrease in intensity and broadening of most peaks.

Figure 4 indicated the refractive index for soluble lens proteins to all the studied groups. The same phenomena in appearing no variation in its value of RI to Group 2 or 3 and a significant difference (increase, $p < 0.05$) observed in Group 4.

Figure 4 refractive index of lens for control (Group 1), hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+11.4 mg/kg/day of atorvastatin (Group 4).

The human eye lens is an oval structure made mostly of protein and water sitting right behind the colored iris and pupil. The lens is consisting of mainly of crystalline proteins linked in a greatly ordered, interactive macro-structure essential that is necessary for

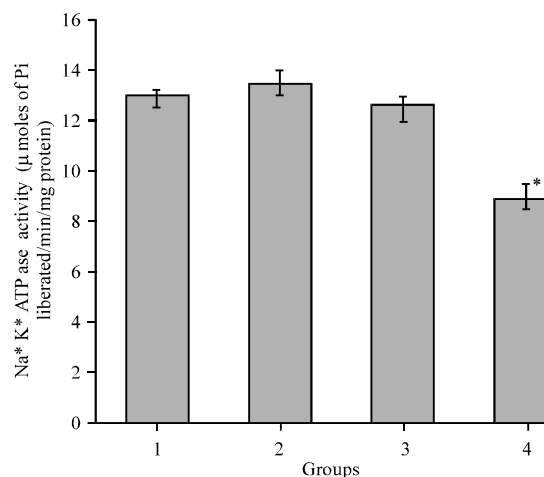


Fig. 1: $\text{Na}^+\text{-K}^+$ ATPase activity of lens membrane for control (Group 1), hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+11.4 mg/kg/day of atorvastatin (Group 4)

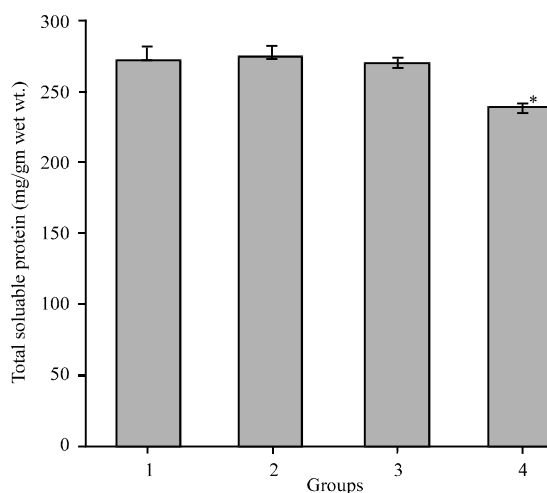


Fig. 2: Total soluble lens proteins for control (Group 1), hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+11.4 mg/kg/day of atorvastatin (Group 4)

lens transparency and refractive index. Any disturbance of intra- or inter-protein interactions will change this gentle structure, revealing hydrophobic surfaces with resulting protein accumulation and cataract formation. Cataract is defined as the clouding of the lens of the eye is the main cause of blindness, affecting tens of millions of people worldwide (Zhao *et al.*, 2015).

Cataract extraction with implantation of an intraocular lens is the most effective approach to avoid loss of vision

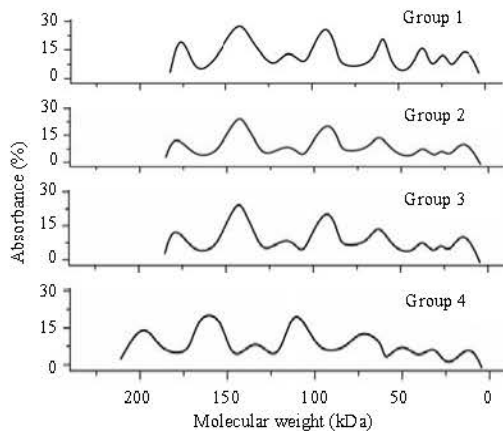


Fig. 3: Electrophoretic pattern of soluble lens proteins for control (Group 1) hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+11.4 mg/kg/day of atorvastatin (Group 4)

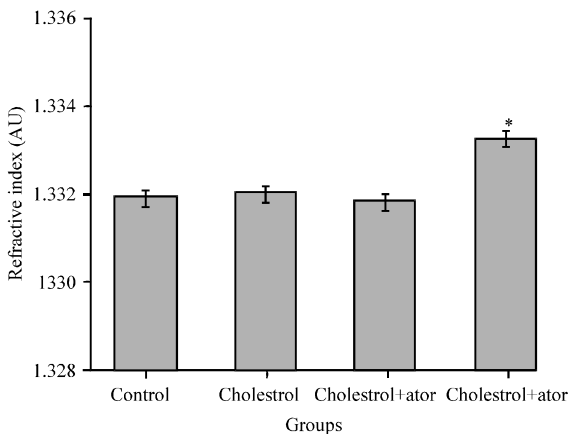


Fig. 4: Refractive index of lens for control (Group 1), hypercholesterolemic (Group 2), hypercholesterolemic+1.14 mg/kg/day of atorvastatin (Group 3) and hypercholesterolemic+ 11.4 mg/kg/day of atorvastatin (Group 4)

from cataract worldwide (Prokofyeva *et al.*, 2013). However, it is important to consider that this treatment approach is quite expensive and risky in terms of post-surgery example capsule opacification, retinal detachment, endophthalmitis, posterior capsular rupture with vitreous loss, vitreous stands to the surgical incision and iris prolapsis through the corneal/limbal incision that may consequently result in permanent loss of vision (Vrensen, 2009). Therefore, priority must be given to the nonsurgical approaches as the best course of prevention of cataract.

Uses of drugs which set back cholesterol biosynthesis can be associated with cataracts in animals and man (Cenedella, 1996). The core of this relationship lies in the lens's need to satisfy its constant requirement for cholesterol by on-site synthesis and preventing this synthesis can lead to a change in the structure of the lens membrane. Lens membrane contains the highest cholesterol content of any known membrane saturation with cholesterol smoothed the phospholipids-bilayer surface which should decrease light scattering and help to maintain lens transparency.

Statin drugs block the function of the liver enzyme that produces cholesterol. Excess cholesterol in blood causes increase of plaque on the arteries lining. That buildup can eventually cause the arteries to narrow or harden. Sudden blood clots found in these narrowed arteries can result in a heart attack or stroke. Liver enzyme also produces CoQ10, so, if you prevent production of cholesterol you also block CoQ10. When free radical damage affects the lens in the eye, it causes it to become opaque as cataracts are being formed. CoQ10 has powerful antioxidant properties and perhaps this is why statins are linked with cataract formation due to the CoQ10 being blocked by this drug (Golomb *et al.*, 2012).

In the current study, the results indicated a relationship between atorvastatin and the total of lens proteins that consider the primary step in cataract formation.

The observed lowering total soluble lens protein is apparently in Group 4 that animal's hypercholesterolemic and treated with 11.4 mg/kg/day with atorvastatin can be clarified by a differential reduction in protein synthesis. This differential effect could be shown by certain differences in the intracellular concentration of Na⁺ and K⁺ ions in the lens; Na⁺ levels noticeably increased and K⁺ decreased. Recent reports show, the decreasing of this intracellular ratio in lens results in a shift of protein synthesis from lower to higher molecular weight species that appeared in electrophoresis mobility of the same group. In view of this information and the outcomes of the current study, we suggest that the development and/or progression of cataract related to treatment by atorvastatin, especially, when needed in high doses.

These data have recommended that high doses of HMG-CoA reductase inhibitors may enhance lenticular exposure to treatment via the aqueous humor by producing an extensive systemic exposure to drug substance. This may cause an increased in the concentration of inhibitor in the outer cortical region of the lens where cholesterol synthesis is vital, thus, resulting in the progress of opacities.

As reported Peter *et al.* (1973) mentioned that desmosterol that a molecule similar to cholesterol amounts is 95% of the plasma sterols and 92% of erythrocyte membrane sterols in treated rats after 5-6 months the action of Na⁺-K⁺ ATPase is elevated in the erythrocyte membranes and sarcolemma that means desmosterol accumulation could be the cause for cataracts. Also recently in agreement with our results. Casula *et al.* (2016) concluded the association of cataract with statin therapy.

Some studies used specialized techniques in relatively small patient populations (Chylack *et al.*, 1993; Schmidt *et al.*, 1990, 1994; Harris *et al.*, 1995) or repetitive slit-lamp examination in large populations (Thorgeirsson, 1996; Laties, 1991) showed that lens opacities occurred with similar rates in the active and placebo groups. Therefore, cataracts are no longer considered a risk of statin therapies and clinical trials are needed to approve or refuse this association.

Statins are used for their positive effect in the cure of patients with known cardiovascular disease. In fact, statin use has proven to noticeably decrease hospital admissions, improve surrogate endpoint outcomes and reduces overall mortality (Foody *et al.*, 2006; Hognestad *et al.*, 2004; Ray *et al.*, 2005). However, several studies prove that statins when used for primary prevention have little effect on cardiovascular (Bates *et al.*, 2009; Bouchard *et al.*, 2007; Perreault *et al.*, 2005; Poluzzi *et al.*, 2008). In addition to consider their possible side effects, long-term adherence to statin poses a potential risk, especially, among individuals without cardiovascular disease. The main use of statins should be closely analyzed, especially, when taking into consideration the possible hazards linked to the usage of statin.

CONCLUSION

There are many natural ways to decrease cholesterol and it is only by addressing our whole diet and lifestyle that we can make enduring changes that will lower cholesterol without any side effects due to drugs. Lens turned out to be reacted to atorvastatin, especially, high doses administrated.

RECOMMENDATION

To identify the exact association, further double-blinded randomize controlled trials must be considered.

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