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Research Article

EFFECT OF STATINS ON NORMAL AND GLUCOSE INDUCED CATARACT IN GOAT LENS

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ABSTRACT

Background of the work: Cataract, a condition in which the lens becomes opacified, is one of the major complications of diabetes. Oxidative damage by the free radicals has been implicated in the pathology of cataractogenesis. Diabetes with dyslipidemia is a common finding where statins have clearly established their role as 1st line drugs.

Objectives: To study the effects of various statins on normal and glucose induced cataract in an *in vitro* model of goat lens.

Methodology and Techniques: Goat lenses obtained from local slaughterhouse were incubated in artificial aqueous humour containing 5.5 mM of glucose or 55 mM of glucose (cataractogenesis) with statins in different concentrations at room temperature for 72 hrs. Opacification of lens was assessed by counting the no. of clear squares when placed over a graph paper before and after 72 hrs of incubation. Biochemical parameters like cholesterol and MDA levels of lens homogenate were estimated. All the data relating to biochemical parameters were compared using one-way ANOVA with post-hoc Dunnet's test.

Results: Glucose induced opacification of lens started 12 hrs post incubation and was completely opacified in 72hrs. Cataractous lenses showed higher MDA levels ($p<0.01$). Lenses treated with Atorvastatin or Simvastatin in concentrations of 15 and 60ng/ml prevented formation and progression of cataract by glucose as evidenced by biochemical parameters.

Conclusion: The anticataract activity of statins may be because of the antioxidant activity as evidenced by lower MDA levels in treated lenses.

Keywords: Cataract, Dyslipidemia, Opacification

INTRODUCTION

Cataract is a major cause of blindness all over the world. It is a condition in which the lens becomes opacified, is one of the major complications of diabetes. Oxidative damage by the free radicals has been implicated in the pathology of catractogenesis [1]. Recent advances in the free radical chemistry have shown considerable changes in therapeutic schedules in the prevention and treatment of cataract secondary to diabetes. On the other hand, diabetes with dyslipidemia is a common finding where statins have clearly established their role as 1st line drugs [2, 3]. Various studies in human and animal models with regard to the effect of statins on cataract have been done but with controversial results [4-7]. Endogenous antioxidant like reduced glutathione is present in high concentration in lens; Also super-oxide dismutase and catalase keep the level of free radicals below toxic levels. In cataractous lenses its concentration is decreased (Graw J et al, 1985). Hence, it's obvious that antioxidants can prevent cataract formation. Besides their cholesterol-lowering effect, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) show many pleotrophic effects, one of which has been suggested is their anticataract effect in a setting of diabetic dyslipidemia. Conversely, these drugs have been demonstrated to induce cataract in experimental studies. Opacity of cornea and lens were seen in dogs treated for 3 months at 30 mg/kg/day and 1 year at 1 and 6 mg/kg/day. The exposure levels at 1 mg/kg in the one year study were comparable to human exposure at 80 mg/day. The clinical association between statin treatment and cataract has not been clearly identified. Current clinical studies have not found direct association between statin treatment and cataracts. In this context, the present study was conducted to elucidate the effect of statins in an in vitro model of experimental cataract [8].

MATERIALS AND METHODS

Materials

Fresh goat eyes were obtained from local slaughterhouse immediately after slaughter and transported to the laboratory at 0-4º C. Glucose and all other chemicals used in this experiment were analytical grade.

Lens extraction and incubation

All the experiments were done with prior permission of the local Animal Ethical Committee. (IAEC). Glucose induced Cataract Model in Goat lens was utilized in the present study as they are easily available locally and can be incubated at room temperature in a suitable medium. The lenses were removed by extra capsular extraction (Figure – 1) and incubated in artificial aqueous humor at room temperature and pH 7.8 for 72hrs. The composition of aqueous humor was as follows: $NaCl_2$- 140 mM, KCl - 5 mM, $MgCl_2$- 2 mM, $NaHCO_3$- 0.5 mM, $NaH(PO_4)_2$- 0.5 mM, $CaCl_2$- 0.4 mM and Glucose-5.5 mM. All the chemicals used were of Laboratory analytical grade. Strict aseptic measures were followed and any possible contamination of the culture media was prevented by adding penicillin 32mg%, streptomycin 250mg% and replacing the aqueous humor with fresh solution every 24hrs. Glucose in a concentration of 55 mM was used to induce cataract[9].

Study drugs and doses: Atorvastatin (Cipla pharmaceuticals – gift sample): - 15 ng/ml and 60 ng/ml & Simvastatin (Cipla pharmaceuticals – gift sample): - 15 ng/ml and 60 ng/ml.

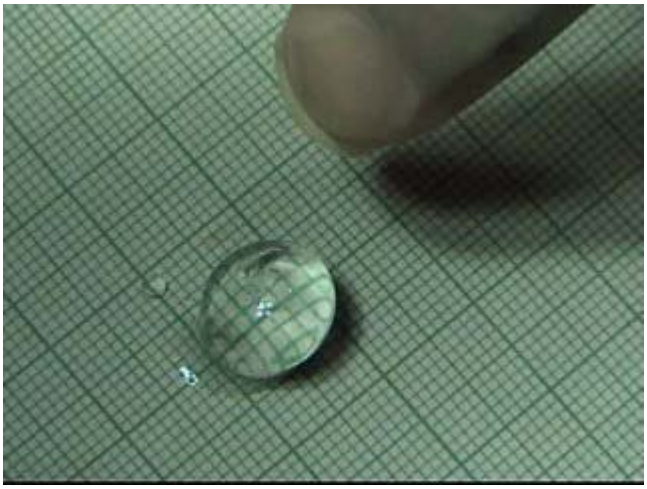
Methodology and Techniques

Opacification of lens was assessed by counting the no. of clear squares before and after 72 hrs of incubation when placed over a graph paper shown in Figure: II. Biochemical parameters like cholesterol and MDA levels of lens homogenate were estimated.

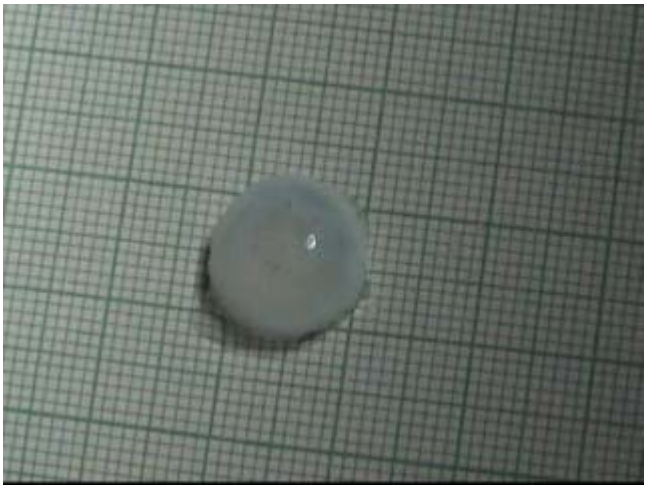
Plan of study: A total of 60 lenses were divided into ten categories (n=6 in each category). Group 1 and Group 2 incubated with 5.5 mM and 55 mM of glucose respectively. Group 3 and Group 4 incubated with atorvastatin (15 & 60 ng/ml) in artificial aqueous humour (Glucose 55 mM). Group 5 and Group 6 incubated with simvastatin (15 & 60 ng/ml) in 55 mM of Glucose. Group 7 and Group 8 incubated with atorvastatin (15 & 60 ng/ml) in artificial aqueous humour (Glucose 5.5 mM). Group 9 and Group 10 incubated with simvastatin (15 & 60 ng/ml) in 5.5 mM of Glucose.

Homogenate preparation: After 72 hrs of incubation, homogenate of lenses was prepared in Tris buffer (0.23M, pH 7.8) containing0.25 × 10-3 M EDTA and homogenate adjusted to 10% w/v. The homogenate was centrifuged at 10,000G at 40C for 1 hr and the supernatant was used for estimation of biochemical parameters.

Opacity evaluation: Lenses were placed on a graph paper with posterior surface touching the paper and the no. of clear squares visible through the lens before and after incubation was counted and used as a measure of lens opacity shown in Figure II.



Before incubation



After incubation

Stastical Analysis

All the data relating to biochemical parameters are expressed as mean ± SD. For evaluating the opacity post incubation the normal & glucose induced cataractous groups were compared using one-way ANOVA with post-hoc LSD test .For evaluating the biochemical parameters the normal as well as glucose induced cataractous groups were compared using one-way ANOVA with post-hoc Dunnet's test taking 5.5 mM of glucose & 55 mM of glucose treated group as controls respectively. $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

In our study incubation of lenses with glucose 55 mM showed that opacification started after 10 hrs at the periphery (posterior) surface and progressively increased towards centre with complete opacification at 72hrs. There was a significant degree of opacification in all the glucose treated lenses shown in Figure III. Lenses incubated in normal aqueous humor (control & atorvastatin) did not show opacification. But, simvastatin in both the doses produced significant opacification compared to the normal control shown in Table: 1(a) & 1(b).

There has been considerable concern with regard to the cholesterol biosynthetic pathway in the ocular lens because, unlike many other tissues, the lens is avascular in nature and therefore has limited accessibility for serum lipoproteins and dietary cholesterol. This pathway plays an important role in maintaining lens integrity and function. *De Vries et al* demonstrated that, in rats aged 20 to 42 days, oral treatment with simvastatin (50 mg/kg per day) decreased the concentration of lens cholesterol by 45% compared with controls[10]. *Gerson et al*. produced sub capsular lenticular opacities in dogs by administering large doses of different HMG-CoA reductase inhibitors such as lovastatin, simvastatin, and other related compounds but, this cataractogenic effect was not found to correlate with lowered levels of lens or serum cholesterol[11]. Also the molecular mechanism involved in cataractogenesis in these situations was poorly understood. Similarly, Hockwin O et al (1991) found no HMG-CoA reductase activity with calf, bovine, dog and rat lenses. Therefore one of the aims of this project was to determine whether use of Atorvastatin or Simvastatin was associated with a change in cholesterol concentration of the goat lens. However our study also did not reveal any statistically significant differences in cholesterol concentration in any groups both in the normal as well as glucose induced cataractous lens that is there was no association between the cholesterol content and opacity in the study groups shown in Table: 2(a) & 2(b).

Many products of lipid per oxidation are not overtly toxic or are the minor products. The important toxicological interest are malondialdehyde (MDA), 4-hydroxynonenal and various 2-alkenals, among them Malondialdehyde is a major reactive aldehyde and is used as an indicator of tissue damage. A significant increase in plasma MDA in high glucose(55mM) group obtained in the present study is consistent with the study by D G Langade et al (2006).The MDA levels were significantly less in the Atorvastatin and Simvastatin treated groups in both the concentrations in the glucose induced cataractous lenses. This observation is in agreement to that of other studies,[12] like Tan JS et al (2007) who concluded that statins use was found to reduce the risk of cataract development by 50%, principally nuclear or cortical cataract subtypes but contradicts other studies like that of Cenedella RJ,(2003) et al who reported that Simvastatin rapidly induced cataracts in young Chbb:Thom (CT) but not Sprague Dawley (SD) or Hilltop Wistar (HW) rats.

There was no significant difference in the MDA levels between the treatments groups in the lens incubated in normal aqueous. The above finding suggests that Atorvastatin and Simvastatin are not cataractogenic by themselves rather they appear to have a preventive role in diabetic cataract in this invitro model.

Table 1(a): Effect of statins on lenticular opacity in glucose induced cataractous goat lens

S. No.	Drug/vehicle	Transparent squares(BD) (mean±sem)	Transparent squares (AD) (mean±sem)
Gr.1	Glucose 5.5nM	44.83±1.97	43.67±2.03
Gr.2	Glucose 55nM	43.00±3.45	5.33±0.84$^{\bullet}$
Gr.3	Glucose55nM+A15	46.00±1.46	32.50±1.98$^{\bullet\bullet}$
Gr.4	Glucose55nM+A60	44.50±1.65	33.33±2.30$^{\bullet\bullet}$
Gr.5	Glucose55nM+S15	41.67±2.99	15.50±0.96$^{\bullet\bullet}$
Gr.6	Glucose55nM+S60	42.67±2.95	8.00±0.26$^{\bullet\bullet}$

Values are expressed as mean±sem, n=6 for each group. $^{\bullet}$ 'p'<0.01 compared to the 5.5nM glucose treated control . $^{\bullet\bullet}$'p'<0.01 compared to the before drug value of each group.

Table 1(b): Effect of statins on lenticular opacity on normal goat lens

S. No.	Drug/vehicle	Transparent squares (BD) (mean±sem)	Transparent squares (AD) (mean±sem)
Gr.1	Glucose 5.5nM	44.83±1.97	43.67±2.03
Gr.7	Glucose5.5nM+A15	42.67±2.95	35.17±2.69
Gr.8	Glucose5.5nM+A60	43.83±2.74	36.00±2.73
Gr.9	Glucose5.5nM+S15	42.50±3.22	29.00±2.29*
Gr.10	Glucose5.5nM+S60	46.50±2.35	7.50± 1.38*

Values are expressed as mean±sem, n=6 for each group * 'p'<0.05 compared to the 5.5nM glucose treated control and also Glucose5.5nM+S15 & S60 treated groups were significantly different from their before drug values.

Table 2(a): Effect of statins on biochemical parameters in glucose induced cataractous goat lens

S. No.	Drug/vehicle	Cholestrol (mean±sem)	MDA (mean±sem)
Gr.1	Glucose 5.5nM	13.64±1.95	4.67±0.92 *
Gr.2	Glucose 55nM	22.24±2.95	17.00±3.83
Gr.3	Glucose55nM+A15	13.83±1.82	2.50±0.81*
Gr.4	Glucose55nM+A60	20.67±3.33	5.33±1.09 *
Gr.5	Glucose55nM+S15	20.75±1.28	3.33±1.50 *
Gr.6	Glucose55nM+S60	18.31±3.16	4.70±0.94 *

Values are expressed as mean±sem, n=6 for each group * 'p'<0.05 compared to the 55nM glucose treated control.

Table 2(b): Effect of statins on biochemical parameters on normal goat lens

S. No.	Drug/vehicle	Cholestrol (mean±sem)	MDA (mean±sem)
Gr.1	5.5nM	13.64±1.95	4.67±0.92
Gr.7	5.5nM+A15	18.57±4.44	3.67±0.56
Gr.8	5.5nM+A60	13.09±1.81	4.00±1.32
Gr.9	5.5nM+S15	22.49±3.06	8.50±2.81
Gr.10	5.5nM+S60	23.82±2.31	8.17±4.00

Values are expressed as mean±sem, n=6 for each group *'p'<0.05 the 5.5nM glucose treated control .

CONCLUSION

Although a wide number of drugs are available today for effective treatment of diabetes associated dyslipidemia, statins are gold standard drugs and the growing evidences of their pleiotropic effects establish their supremacy over other available lipid lowering agents, as they are most effective, best tolerated and can provide additional benefits like the antioxidant effect in diabetic cataract as evidenced in the present *in vitro* study. Even though, in contrast to other workers' results, we did not find any activity of HMG-CoA-reductase in our experiments with goat lenses and also their incubation in a normal lens does not seem to be associated with an increased risk of cataract, the preventive role of statins in cataract was proved. With the increasing trend for initiating statin therapy among diabetics with or without hyperlipidemia, the need to assess the effect of long-term and high dose exposure on eye in various other animal models as well as clinical trials including post marketing surveillances remains.

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