Indian J. Environ. Toxicol. 10(1) : 26-29, 2000

Protein profiles in the pre-cataractous and cataractous stages of selenite cataract in rats

Joe Prasad Mathew, V.C.Thomas and Issac Thomas*
Research Division, Post Graduate Department of Zoology
St. Berchmans' College, Changanacherry
Kerala - 686 101, India

Abstract

Selenite cataract is an easily available experimental model to study cataractogenesis and can be easily produced within a week by administering sodium selenite in microgram quantities subcutaneously. The present study was conducted after administering various multiple low dosages and single high dosages of sodium selenite in 8/10 days old rat pups to analyse dose dependent variations in the protein profile of the lens. Biochemical estimations of various protein fractions in the lens and total proteins in the aqueous humour and blood were measured during different stages (nuclear, mature, hypermature etc.) of selenite cataract. The results showed an increase in the insoluble fractions with increase in opacity. The day to day analysis revealed that the action of selenite is sudden, since even after 24 hrs. considerable changes in the protein fractions were observed. The reason for alteration in the protein fractions was discussed in relation to the possible oxidative action of selenite with lens proteins.

Key words : Wistar rats, lens, proteins, selenite, cataract

Introduction

Both qualitative and quantitative changes occur in lens proteins, including insolubilization of crystallin proteins in most of the cataractous lenses in man and animal models (Avarachan, 1985 and Zigler, 1990). Since the lens should have proper constituents and structure, in order to maintain its transparency, an imbalance of protein contents among soluble and insoluble fractions, particularly an increase in the insoluble fraction, would reduce the transparency of the lens (Bettelheim, 1985). In human and selenite induced cataract, a number of biochemical changes occur (Rawal *et al*., 1982; Spector, 1984; Avarachan, 1985). Approximately 90% of the highly concentrated cellular proteins in the mammalian lenses consist of two families of water-soluble lens crystallins, called α-crystallin and β/γ-crystallin (Harding, 1991). There is availability of literature on protein profiles in selenite cataract model but a paucity exists on dose-dependent studies.The present study mainly has been taken up to study the dose-dependent changes occuring in protein profiles in low and high dose induced selenite cataract.

Material and methods

The induction and various stages of selenite cataract were produced in both sexes of Wistar rat pups using multiple low dosages of 14.4, 19.2, 23, and 27.8 µg. sodium selenite/~20 g. rat pups subcutaneously, and single high dosages of 32, 40, 56 and 64 µg. /~20 g. rat pups subcutaneously.

For multiple low dosages, five doses were given starting on day eight *post partum* and for high dosages, a single dose was given on 10th day *post partum* (Table 1).

Table 1. Experimental design

Group	No. of animals in each group	Dosage in µg.	No. of Doses	Administered on (postpartum days)	Stage of cataract extraction and protein analysis
Multiple low dosage group	8 8 8 8	14.4 19.2 23 27.8	5 5 5 5	8 8 8 8	Nuclear stage
Single high dosage group	8 8 8 8	32 40 56 64	1 1 1 1	10 10 10 10	Nuclear, Mature, Hypermature stages
Day to Day group	40 (8/day)	40	1	10	1 to 5 day after selenite administration
Controls: For each group, separate control was used for which an equal amount of double distilled water was administered subcutaneously in place of sodium selenite. In all groups 8 control rat pups were used as in experimental cases.					

Biochemical analyses were carried out in different experimental groups, and their controls. Nuclear stage of

cataract produced by low dosages, nuclear, mature and hyper mature stages of cataract produced by high dosages, along with their age-related controls were used for the biochemical study (Mathew *et al.*, 1996-97). Besides this, a first to fifth day biochemical analysis of the lens with their controls, (on each day) after the administration of 40 µg. sodium selenite/rat pup, was also carried out. At the required time, the rats were sacrificed and the complete eye lenses were dissected out immediately by the posterior approach. The weight of the lens samples were taken on a balance sensitive to 0.01 mg. The blood was collected wherever necessary, from the jugular vein into a tube containing anticoagulant EDTA, within two minutes after sacrificing the rats. The aqueous humour samples were collected wherever necessary by the method of Kinsey *et al*. (1963) using a micro syringe. Total, soluble and insoluble lens proteins, aqueous humour and blood proteins were estimated by the method of Lowry *et al*. (1951).

Results and discussion

This study elicited a reduction in the protein content in the water-soluble fraction and an increase in the insoluble fraction in all the experimental groups studied (Tables 2, 3 and 4). However, the magnitude of this decrease or increase varied depending upon the dosage and the advancement of the cataractous stages. The total protein showed a significant decrease in the low dosage group. In the high dosage group, a highly significant drop in the total protein concentration was observed in the hyper mature stage. A significant rise in insoluble protein was seen even in the nuclear stage of the cataract, produced by both the low and high dosage groups; and this increased more in the mature and hyper mature stages with high dosages, with subsequent decrease in the soluble protein concentration.

Table 2.Protein profile (±) in the lens, aqueous humour and blood samples of selenite cataract with multiple low dosage of sodium selenite (n=8)

Sample/Parameters	**Nuclear stage** (25-30 day *post partum*) Dosage in µg/~20 mg rat pup				
	Control	14.4	19.2	23	27.8
Lens–Total protein mg/mg wet wt.	0.359 ±0.003	0.330** ±0.003	0.325** ±0.002	0.322** ±0.004	0.314** ±0.004
Lens–Soluable protein mg/mg wet wt.	0.291 ±0.003	0.198** ±0.004	0.198** ±0.002	0.192** ±0.002	0.184** ±0.005
Lens–Insoluable protein mg/mg wet wt.	0.075 ±0.003	0.138** ±0.004	0.134** ±0.003	0.133** ±0.004	0.133** ±0.003
Aqueous humour–Total protein mg/ml	41.61 ±0.79	40.45^{NS} ±0.78	40.32^{NS} ±0.77	39.56^{NS} ±0.59	39.25^{NS} ±0.63
Blood–Total protein mg/ml	81.77 ±1.21	78.36** ±0.49	78.43** ±0.49	79.33** ±0.39	79.74** ±0.48

Values marked in astericks differ significantly from the corresponding control value (P < 0.01).

All

The observation on each day after selenite injection showed a significant rise (Table 4) in the lens insoluble protein fraction by 72 hrs. At the same time the lens soluble proteins showed a significant decrease even after 24 hrs. The total protein and blood protein also decreased significantly at this stage. The

Table 3. Total protein, insoluble protein, and soluble protein in the lens; total protein concentration in the aqueous humour and blood samples of selenite cataract with various high dosages of sodium selenite (n=8)

Nuclear stage Samples/ parameters	Dosage in µg/~20 mg rat pup				
	Control	32	40	56	64
Lens–Total protein mg/mg wet wt.	0.361 ±0.003	0.340** ±0.003	0.333** ±0.005	0.324** ±0.004	0.323** ±0.004
Lens–Soluable protein mg/mg wet wt.	0.282 ±0.005	0.180** ±0.002	0.179** ±0.008	0.178** ±0.008	0.175** ±0.004
Lens–Insoluable protein mg/mg wet wt.	0.070 ±0.002	0.137** ±0.001	0.138** ±0.001	0.140** ±0.001	0.143** ±0.002
Aqueous humour–Total protein mg/ml	42.81 ±1.07	42.38^{NS} ±0.95	41.99^{NS} ±0.56	41.36^{NS} ±0.73	40.47^{NS} ±0.65
Blood–Total protein mg/ml	85.19 ±0.18	83.69** ±0.44	82.35** ±0.33	82.14** ±0.13	82.08** ±0.07

Mature stage Samples/param eters	Dosage in µg/~20 mg rat pup				
	Control	32	40	56	64
Lens–Total protein mg/mg wet wt.	0.370 ±0.002	0.331** ±0.006	0.329** ±0.008	0.325** ±0.008	0.324** ±0.003
Lens–Soluable protein mg/mg wet wt.	0.287 ±0.002	0.150** ±0.005	0.149** ±0.003	0.144** ±0.004	0.142** ±0.003
Lens–Insoluable protein mg/mg wet wt.	0.078 ±0.002	0.162** ±0.002	0.163** ±0.001	0.165** ±0.001	0.167** ±0.002
Aqueous humour–Total protein mg/ml	44.90 ±0.57	38.12** ±0.20	37.69** ±0.50	37.82** ±0.53	36.78** ±0.33
Blood–Total protein mg/ml	87.75 ±0.45	82.31** ±0.39	81.85** ±0.20	81.27** ±0.14	80.24** ±0.12

Hyper mature stage Samples/param eters	Dosage in µg/~20 mg rat pup				
	Control	32	40	56	64
Lens–Total protein mg/mg wet wt.	0.374 ±0.003	0.244** ±0.008	0.240** ±0.006	0.230** ±0.005	0.225** ±0.006
Lens–Soluable protein mg/mg wet wt.	0.291 ±0.003	0.074** ±0.010	0.074** ±0.008	0.071** ±0.003	0.070** ±0.002
Lens–Insoluable protein mg/mg wet wt.	0.070 ±0.001	0.184** ±0.001	0.192** ±0.002	0.194** ±0.001	0.196** ±0.001
Aqueous humour–Total protein mg/ml	44.90 ±0.18	39.78** ±0.62	38.78** ±0.25	38.05** ±0.21	36.48** ±0.19
Blood–Total protein mg/ml	88.78 ±0.39	81.49** ±0.16	80.81** ±0.18	80.28** ±0.14	80.05** ±0.09

Values marked in astericks differ significantly from the corresponding control value (P < 0.01).

reduction in the soluble protein fraction and the increase in insoluble protein fraction is due to the oxidation of proteins by the selenite. In animals, selenite intereact with proteins in many ways.

r

Table 4. Protein profile in the lens, aqueous humour and blood of a 40mg. sodium selenite dosage from 1st to 5th day after administration (n=8)

Dosage	40 µg./~20 g. rat pup									
Sample/ Parameters	Control	1st day	Control	2nd day	Control	3rd day	Control	4th day	Control	5th day
Lens–Total protein mg./mg. wet wt.	0.385 ±0.004	0.383^{NS} ±0.002	0.385 ±0.003	0.373** ±0.004	0.386 ±0.002	0.359** ±0.003	0.387 ±0.002	0.352** ±0.004	0.391 ±0.003	0.353** ±0.002
Lens–Soluable protein mg./mg. wet wt.	0.242 ±0.002	0.236** ±0.001	0.245 ±0.002	0.234** ±0.001	0.246 ±0.002	0.203** ±0.003	0.252 ±0.001	0.182** ±0.001	0.254 ±0.002	0.174** ±0.003
Lens–Insoluable protein mg./mg. wet wt.	0.141 ±0.001	0.140^{NS} ±0.002	0.143 ±0.002	0.144^{NS} ±0.004	0.146 ±0.002	0.161** ±0.002	0.146 ±0.002	0.171** ±0.003	0.149 ±0.001	0.183** ±0.002
Aqueous humour–Total protein mg./ml.	41.26 ±0.01	40.26^{NS} ±0.01	41.30 ±0.01	40.16^{NS} ±0.02	41.38 ±0.02	40.13^{NS} ±0.01	41.59 ±0.02	40.08^{NS} ±0.04	41.90 ±0.02	40.03^{NS} ±0.01
Blood–Total protein mg./ml.	84.14 ±0.01	83.56** ±0.01	84.84 ±0.04	83.62** ±0.01	84.90 ±0.08	83.85** ±0.09	85.05 ±0.19	83.24** ±0.05	85.28 ±0.04	83.20** ±0.02

All values are Mean ± S.D. for groups of eight rats and those marked with asterisks differ significantly from the corresponding control value ($P < 0.01$).

Selenite react to form selenotrisulfides (R–S–Se–S–R), and oxidise sulfhydryls of protein (PSH) (Sunde, 1990).

The reactions are important since oxidation of the critical membrane proteins and enzyme sulfhydryl groups by selenite, alter the membrane property of the lens epithelium and ion homeostasis. This eventually leads to liquefaction and opacity. Bunce and Hess (1981) found a decrease in the NADPH concentration 24 hrs after selenite administration, because of the reduced co-operative activity of the important glycolytic enzymes, which are the suppliers of NADPH, as the source of reducing equivalents to convert GSSG to GSH.

Selenite is incorporated into proteins as selenoaminoacids, such as selenocystine (Shearer *et al*., 1992). Selenite is first reduced to selenide (SeH^-), followed by incorporation of selenide into P-serinyl-tRNA, to form selenocysteinyl tRNA for protein synthesis.

$$SeO_3^{-2} + 4\ PSH \rightarrow PssP + Ps–Se–sP$$
$$Ps–Se–sP + O_2 \rightarrow SeO_3^{-2} + Ps\ sP$$

$$SeO_3^{-2} \rightarrow Se\ H^-$$
$$P–Ser–tRNA + SeH^- \rightarrow Se–Cys–tRNA \rightarrow \text{selenoprotein.}$$

This results in the incorporation of much of selenium into the lens. According to Shearer *et al*. (1984), the identity of selenoaminoacids in α- and β-crystallins are unknown, and γ-crystallin showed very little incorporation into an unusual acidic amino acid.

The following order of events were postulated by Shearer *et al*. (1992) to occur during the cataract formation in the selenite model. Selenite may oxidises critical sulfhydryl groups, leading to the inactivation of Ca^{2+}–ATPase or opening of ion channels in the epithelial membranes. Calcium influx then causes activation of the calcium-dependent protease Calpain II, which partially degrades α and β-crystallins. These partially degraded crystallins expose the hydrophobic regions, and the thiol groups, which then oxidised by selenite itself or by other reactive oxygen species to form insoluble aggregates. The partially degraded β-crystallins become insoluble, and scatter light (David and Shearer, 1993; Shearer *et al*., 1995).

The studies carried out by Mathew (1998) confirmed that insolubilization and aggregation of the lens protein crystallins, contribute to opacification, Matsushima *et al*. (1997) suggested that degradation of non-crystallin cyto-skeletal proteins are important during the early stages of opacification in the selenite cataract.

A significant reduction in the total protein in the hypermature stages with high dosages (Table 3) is due to a reduction in the protein synthesis as a result of the selenite action. Selenite is able to retard protein synthesis by direct inhibition of elongation factor 2 by selenodiglutathione (Vernie *et al*., 1975), and by inactivation of the initiator met-tRNA binding factor (Safer *et al*., 1980). Selenodiglutathione is the reaction product of GSH and selenite (Kice *et al*., 1980). The decrease in the total protein concentration in part is due to an enhanced protein degradation as a result of oxidation.

The protein concentration in the aqueous humour was interesting. Its concentration slightly decreased in the early (1–5) days, after injection (Table 4) and with low dosage nuclear cataract (Table 2). This is due to the degradation of protein by oxidative insult or due to decreased synthesis. However, in the advanced stages of cataract with high dosages (Table 3), there occurs an increase in the total protein concentration when compared to the controls. This denotes a plausible leakage of proteins from the lens due to membrane degradation. Watanabe and Shearer (1989) and Watanabe *et al*. (1990) observed a leakage of α, β and γ-crystallins from the lens into the aqueous and vitreous humours during selenite induced nuclear and cortical cataract. In rats, they have observed a 10-fold increase in crystallin concentrations in the aqueous, and about a 20-fold increase in crystallins in the vitreous, during mature selenite cataract.

The total protein concentration in the blood showed a significant decrease in the hyper mature stage with the high dosages (Table 3).This points towards the fact that higher concentrations of selenium in the blood have some negative impact upon the protein profile. Burk (1974) suggested that selenium showed affinity to certain plasma proteins. A decline in the total serum protein in humans exposed to selenium factory conditions was also reported (Mathew, 1998). Read *et al.* (1990) showed that selenoprotein P is the predominant form of bound selenium in serum, and glutathione peroxidase accounts for most of the remaining bound selenium. According to Shearer *et al.* (1992), the function of selenoprotein P is unknown, but assumed to be antioxidative because of its high content of thiol and selenol groups. Besides this, Shearer *et al.* (1984) reported that other than uptake into crystallins, large amounts of ^{75}Se were taken up into small amounts of unknown soluble proteins. Thus the loss of total blood protein could be due to the substitution of large amounts of selenium into these proteins. These biochemical changes in the blood and aqueous humour worsen the pathological condition of the cataractous lens since the lens depends on them for their nourishment through blood-aqueous-lens barriers and gap junctions (Thomas, 1987).

In conclusion, it can be stated that a change in protein profiles starts in the precataractous stages itself and by 72 hrs a significant increase in lens insoluble protein was noticed. By the progression of the cataract a highly significant drop in the soluble fraction of protein is noticed as against an increase in insoluble protein fraction, which results in complete opacification of the transparent lens. The above changes are mainly because of lens protein oxidation by selenite. However there is no dose dependency for these changes except that a high dose can produce the cataractous changes in a shorter time (Mathew *et al.*, 1996-97). Thus the study provided a better understanding about the protein changes in the precataractous and cataractous stages of selenite cataract model.

Acknowledgement

One of us (J. P. M) is thankful to Mahatma Gandhi University, Kerala, for the award of Junior Research Fellowship.

References

Avarachan, P. J. 1985. Protein profile in the progressive experimental cataract. *Ind. J. Ophthalmol.,* **33**(5): 303-308.

Bettleheim, F. A. 1985. Physiological basis of lens transparency. In: *The Ocular Lens,* Maisel H. (Ed.) Marcel Dekker, New York, pp 265-300.

Bunce, G. E. and Hess, J. L. 1981. Biochemical changes associated with selenite induced cataract in the rat. *Exp. Eye Res.*, **33**: 505.

Ireland, M. and Maisel, H. 1984. Evidence for a calcium activated protease specific for lens intermediate filaments. *Curr. Eye Res.,* **3**: 423-429.

Kinsey, V. E. and Reddy, D. V. N. 1963. Studies on the crystalline lens. X. Transport of amino acids. *Invest. Ophthalmol.*, **2**: 229.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurements with the folin phenol reagent. *J. Biol. Chem.,* **193**: 263.

Harding, J. 1991. Biochemistry, epidemiology and pharmacology. In: *Cataract,* Chapman and Hall, New York, U.S.A.

Mathew, J. P., Thomas, V. C. and Issac Thomas. 1997. Cataractogenic potency of a heavy metal selenite in Wistar rats. *J. Zool. Soc. Kerala*, **7**: 51-60.

Mathew, J. P. 1998. Studies on histomorphological and biochemical aspects of experimental cataract: Chronic and acute dosage selenite cataract and impact of vitamin-E in Wistar rats, Ph. D. Thesis, Mahatma Gandhi University, Kerala.

Matsushima, H., David, L. L., Hiraoka, T. and Clark, J. L. 1997. Loss of cytoskeletal proteins and lens cell opacification in the selenite cataract model. *Exp. Eye Res.,* **64**: 387-395.

Rawal, U. M., Khamar, B. M. and Gandhi, D. N. 1982. Human ageing lens-A quantitative study: DNA, RNA and total soluble protein in normal and cataractous lenses. *Ind. J. Ophthalmol.,* **31**: 258-261.

Read, R., Bellew, T., Yang, J.-G., Hill, K. E., Palmer, I. S. and Burk, R. F. 1990. Selenium and amino acid composition of selenoprotein P, the major selenoprotein in rat serum. *J. Biol. Chem.,* **265**: 17899-17905.

Safer, B., Jagus, R. and Crouch, D. 1980. Indirect inactivation of eukaryotic initiation factor 2 in reticulocyte lysate by selenite. *J. Biol. Chem.,* **255**: 6913-6917.

Shearer, T. R., Ridlington, J. W., Goeger, D. E. and Whanger, P. D. 1984. Uptake of ^{75}Se in cataractous rat lens proteins. *Biol. Trace Element Res.,* **6**: 195-205.

Shearer, T. R., David, L. L., Anderson, R. S. and Azuma, M. 1992. Review of selenite cataract. *Curr. Eye Res.,* **11**(4): 357-369.

Spector, A. 1984. The search for a solution to senile cataracts. *Invest. Ophthalmol. Vis. Sci.,* **25**: 130-146.

Sunde, R. A. 1990. Molecular biology of selenoproteins. *Annu. Rev. Nutr.*, **10**: 451-474.

Thomas, I. 1987. Studies on blood-aqueous-lens barriers in vertebrates, Ph.D. Thesis, Gujarat University.

Vernie, L. N., Bont, W. S., Ginjaar, H. B. and Emmelot, P. 1975. Elongation factor 2 as a target of reaction product between sodium selenite and glutathione in inhibiting of amino acid incorporation *in vitro*. *Biochim. Biophys. Acta,* **414**: 283-292.

Watanabe, H., Komoto, M., David, L. L. and Shearer, T. R. 1990. Changes in crystallin concentrations in rat aqueous and vitreous humors after selenium induced reversible cortical cataract. *Jap. J. Ophthalmol.,* **34**: 472-478.

Zigler, J. S., Jr. 1990. Animal models for the study of maternity–Onset and hereditary cataract. *Exp. Eye Res.,* **50**: 651-657.