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IONIC STATE AND ATPASE ACTIVITY DURING SELENITE CATARACTOGENESIS IN WISTAR RATS.

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Selenite cataract is an experimental model cataract which can be produced by administering Sodium selenite in microgram quantities subcutaneously in suckling rat pups. During the present study, Sodium, potassium, calcium and magnesium in the lens were measured at various stages after subcutaneous selenite administration in multiple low (Chronic) and single high (acute) dosages in Wistar rats. Since selenite is a potent oxidant of membrane SH groups, the activity of the main transport enzyme ATPases were determined along with the ion analysis to understand the role of oxidative damage and the alteration of membrane permeability and cation transport in the rat lens during cataractogenesis.

INTRODUCTION

In many cataract lenses, Na ion concentrations are higher than normal(Mercantionio et al 1980; Duncan and Jacob 1984). A similar increase in Na and Ca ion concentration was observed with selenite cataract in Sprague Dawley rats (Bunce and Hess 1981; Shearer et al 1992) in rabbit lenses *in vitro* (Hightower and Mc Cready 1991), and in Wistar rats (Mathew 1998). The small concentration gradient of Na ions in the lens (Paterson 1969; Garner et al 1986), that opposes the lens protein gradient would appear to be necessary for lens clarity (Veretout et al 1989). During *in vitro* studies when this lens gradient was disturbed either by refrigeration (Paterson 1969) or by Na pump inhibition (Spector 1984), opacification results. The gradient is such that Na ion concentration is highest in the posterior region, and lowest in the nuclear and anterior region of the bovine lenses (Garner 1994).

ATPases are important group of enzymes which are controlling the energy metabolism of the body, and in cation transport. ATP is converted to ADP and inorganic phosphate to release energy to be used up in the metabolism of lens in the presence of ATPases. Na-k-ATPase was confined exclusively to the lateral membranes of the epithelial cells. Its presence was also observable on the apical region of the epithelial cell membranes, and on the membrane of cortical fibres located in the anterior pole (Unakar and Tsui 1980). The present investigation was done to assess the ionic imbalance and ATPase activity after various dosages of selenite administration.

MATERIALS AND METHODS

The induction and various stages of selenite cataract were produced in both sexes of Wistar rat pups using multiple low (chronic) dosages of 14.4, 19.2, 23, and 27.8 µg. sodium selenite/~20 g. rat pups subcutaneously, and single high (acute) dosages of 32, 40, 56 and 64 µg./~20 g. rat pups subcutaneously. For chronic dosages, five doses were given starting on day eight *post partum* and for acute dosages, a single dose was given on 10th day *post partum*.

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. 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 was taken on a balance sensitive to 0.01 mg. The analysis of potassium and sodium was done using Flame photometer (Duncan and Bushell 1975), calcium and magnesium were

analysed by atomic absorption spectrophotometer (Duncan and Bushell 1975) and the ATPase activity by the method of Quinn and White (1968). Statistical analysis was performed using ANOVA and student's t-test.

RESULTS

The result obtained in the present study revealed that the selenite had altered the ion homeostasis. A significant increase in sodium and calcium, and a significant decrease in potassium and magnesium, were observed in the nuclear cataract stages. With the advanced stages, a highly significant increase or decrease was observed with all the dosages (Tables 2 and 3). The day-to-day observation revealed a progressive increase in sodium and calcium, during the first two days, followed by a slight decrease and again an increase in the nuclear stage (Table 4). But potassium and magnesium showed a progressive decline after selenite administration. All the changes were found dosage-dependent.

Table I: Experimental Design

Group	**No. of animals in each group**	**Dosage in mg**	**No. of doses**	**Administered on (post partum days)**	**Stage of cataract extraction and Ion analysis**
Multiple low dosage group	8	14.4	5	8	Nuclear stage
	8	19.2	5	8	
	8	23	5	8	
	8	27.8	5	8	
Single high dosage group	8	32	1	10	Nuclear, Mature and Hyper mature stage
	8	40	1	10	
	8	56	1	10	
	8	64	1	10	
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.					

Adenosine triphosphatase (ATPase) showed a progressive decline in the activity after selenite administration in all the experimental groups (Tables 2,3 and 4). A notable decrease was observed from the third day after selenite administration with the 40 µg. dosage (Table 4). With all the high and low dosage, the decrease was gradual and dosage-dependent in the nuclear, mature and hypermature stages (Tables 2 and 3). A significant decrease was observed with the hypermature stage.

DISCUSSION

The observed results indicate that cation pump had deteriorated progressively depending upon the dosage of selenite administered. Inhibition of the lens Na -K- ATPase by selenite, seemed to be

plausible, since the activity of purified Na-k-ATPase was inhibited by selenite, even though a high concentration of selenite is needed (Burgad and Rathbun 1986),.

The fact that a high concentration of GSH could partially reverse selenite inhibition suggested oxidation is involved (Hightower and Mc Cready 1994). Mathew (1998) observed that the GSH concentration decreased progressively in selenite cataract. A significant reduction was observed even one day after selenite administration. Since selenite was reported to have high affinity to SH groups, a possible role for oxidation of cation pump enzymes cannot be ruled out. Selenite could inactivate Na-K-ATPase by the oxidation of critical SH groups. Glutathione is necessary to prevent the oxidation of critical thiol group on Ca^{+2} ATPase enzyme, and that disturbances in intracellular thiol and calcium ion homeostasis, would lead to cytoskeletal abnormalities and membrane blebbing (Jones et al 1983). Oxidation of Ca-ATPase in lens epithelia of rat lenses which developed *in vivo* selenite cataract (Wang et al 1993). It is also reported that in the selenite-treated rabbit lens oxidation of Ca-ATPase occurred, although the degree of damage depended upon the method of presentation of selenite to the Ca-ATPase molecule (Hightower and Mc Cready 1991b). Calcium cataract is induced in young animals *in vitro* by culturing in a medium containing elevated levels of calcium (Bettelheim et al 1995). Calcium activates calpain II, among others, which preferentially degrades vimentin, a cytoskeletal element (Lorand et al 1985). The degradation of vimentin has a special optical consequence. If the cytoskeleton is birefringent, its removal from the fibre cells can cause optical anisotropy fluctuations, and, as a consequence, turbidity (Bettelheim 1978). In selenite cataract the oxidation of critical membrane SH groups, would lead to impaired calcium homeostasis (Shearer et al 1992). An elevated calcium level would lead to the activation of calcium dependent proteolytic enzyme–calpain, and this would result in the proteolysis of crystallins–especially β-crystallin polypeptides.

Table II: Sodium, potassium, magnesium, calcium contents and adenosine triphosphatase activity in the lens samples of selenite cataract with multiple low (chronic) dosage of sodium selenite (n=8)

Parameters	Nuclear stage Dosage in mg./~20 g. rat pup				
	Control	**14.4**	**19.2**	**23**	**27.8**
Sodium % dry wt.	0.1964 ±0.0012	0.1980** ±0.0004	0.1984** ±0.0002	0.1989** ±0.0005	0.1991** ±0.0003
Potassium % dry wt.	0.5638 ±0.0014	0.5387** ±0.0070	0.5357** ±0.0053	0.5272** ±0.0048	0.5225** ±0.0013
Magnesium % dry wt.	0.0980 ±0.0007	0.0190** ±0.0004	0.0185** ±0.0003	0.0180** ±0.0004	0.0177** ±0.0005
Calcium % dry wt.	0.0039 ±0.0005	0.0042^{NS} ±0.0004	0.0043* ±0.0003	0.0043* ±0.0002	0.0049** ±0.0003
Adenosine triphospha-tase (ATPase) µM ip formed/100 mg. wet wt./h.	4.29 ±0.08	4.06** ±0.04	3.97** ±0.03	3.90** ±0.03	3.78** ±0.05

** P<0.01, * P<0.05, NS - Not significant, All values are Mean ± S. D.

As a supportive factor to enzyme oxidative damage, it is reported that selenite reduced the membrane SH groups in the epithelium of selenite-treated lenses (Hightower and Mc Cready 1991). Thus the accumulating evidences suggest that the initial loss of transport capacity is the oxidative inactivation of the ATPase enzymes.

Another reason for the altered cation transport is due to the decreased synthesis of ATPase enzymes, due to lack of viable cells that are capable of producing these enzymes. The enzyme is a component of the membrane proteins, which could be synthesised at a reduced rate. Inhibited

Table III: Sodium, potassium, magnesium, calcium contents and adenosine triphosphatase activity in the lens samples of selenite cataract with various high (acute) dosages of sodium selenite (n=8)

Nuclear stage	**Dosage in mg./~20 g. rat pup**				
Parameters	**Control**	**32**	**40**	**56**	**64**
Sodium % dry wt.	0.1960 ±0.0012	0.1994** ±0.0008	0.2064** ±0.0035	0.2258** ±0.0036	0.2336** ±0.0022
Potassium % dry wt.	0.5650 ±0.0022	0.5170** ±0.0015	0.5138** ±0.0019	0.5052** ±0.0029	0.5021** ±0.0009
Magnesium % dry wt.	0.0984 ±0.0055	0.0171** ±0.0004	0.0172** ±0.0011	0.0167** ±0.0012	0.0160** ±0.0005
Calcium % dry wt.	0.0036 ±0.0004	0.0047** ±0.0006	0.0050** ±0.0007	0.0056** ±0.0005	0.0066** ±0.0006
Adenosine triphosphatase (ATPase) µM ip formed/100 mg. wet wt./h.	4.24 ±0.04	3.74** ±0.06	3.70** ±0.05	3.62** ±0.06	3.46** ±0.05
Mature stage	**Dosage in mg./~20 g. rat pup**				
Parameters	**Control**	**32**	**40**	**56**	**64**
Sodium % dry wt.	0.1948 ±0.0009	0.2559** ±0.0024	0.2873** ±0.0029	0.3558** ±0.0033	0.3664** ±0.0026
Potassium % dry wt.	0.5836 ±0.0012	0.1050** ±0.0013	0.0937** ±0.0015	0.0847** ±0.0025	0.0753** ±0.0018
Magnesium % dry wt.	0.0217 ±0.0004	0.0137** ±0.0029	0.0103** ±0.0011	0.0097** ±0.0009	0.0088** ±0.0007
Calcium % dry wt.	0.0036 ±0.0010	0.0091** ±0.0006	0.0105** ±0.0009	0.0148** ±0.0030	0.0155** ±0.0027
Adenosine triphosphatase (ATPase) µM ip formed/100 mg. wet wt./h.	4.58 ±0.06	3.28** ±0.06	3.15** ±0.05	3.14** ±0.06	2.94** ±0.05
Hyper mature stage	**Dosage in mg./~20 g. rat pup**				
Parameters	**Control**	**32**	**40**	**56**	**64**
Sodium % dry wt.	0.1929 ±0.0012	0.3510** ±0.0096	0.3633** ±0.0052	0.4057** ±0.0029	0.4155** ±0.0023
Potassium % dry wt.	0.5946 ±0.0019	0.0864** ±0.0016	0.0751** ±0.0023	0.0641** ±0.0013	0.0559** ±0.0020
Magnesium % dry wt.	0.0256 ±0.0008	0.0065** ±0.0005	0.0067** ±0.0004	0.0053** ±0.0004	0.0057** ±0.0004
Calcium % dry wt.	0.0033 ±0.0006	0.0125** ±0.0007	0.0151** ±0.0005	0.0163** ±0.0007	0.0185** ±0.0006
Adenosine triphosphatase (ATPase) µM ip formed/100 mg. wet wt./h.	4.90 ±0.05	3.09** ±0.05	2.74** ±0.07	2.53** ±0.05	2.10** ±0.05

** P< 0.01, All values are Mean ± S.D.

Table IV: Sodium, potassium, magnesium, calcium contents and adenosine triphosphatase activity in the lens of a 40 µg. sodium selenite dosage from 1st to 5th day after administration (n=8)

Parameters	Dosage (40 mg./~20 g. rat pup)									
	Control	**1st day**	**Control**	**2nd day**	**Control**	**3rd day**	**Control**	**4th day**	**Control**	**5th day**
Sodium % dry wt.	0.615 ±0.002	0.770** ±0.003	0.507 ±0.001	0.642** ±0.004	0.415 ±0.004	0.489* ±0.003	0.281 ±0.004	0.302** ±0.003	0.236 ±0.005	0.315** ±0.001
Potassium % dry wt.	1.146 ±0.004	1.436** ±0.004	1.117 ±0.002	1.175** ±0.002	1.070 ±0.002	1.149** ±0.002	0.973 ±0.002	0.968* ±0.003	0.936 ±0.001	0.954* ±0.007
Magnesium % dry wt.	0.085 ±0.004	0.071** ±0.002	0.067 ±0.001	0.047** ±0.001	0.057 ±0.002	0.058^{NS} ±0.001	0.054 ±0.004	0.052^{NS} ±0.001	0.046 ±0.002	0.042* ±0.001
Calcium % dry wt.	0.037 ±0.002	0.093** ±0.004	0.035 ±0.002	0.087** ±0.005	0.036 ±0.002	0.047** ±0.004	0.021 ±0.004	0.026** ±0.001	0.015 ±0.002	0.025** ±0.001
Adenosine triphos-phatase (ATPase) µM ip formed/100 mg. wet wt./h.	3.93 ±0.02	3.92^{NS} ±0.01	3.96 ±0.02	3.91** ±0.02	4.06 ±0.03	3.84** ±0.09	4.12 ±0.02	3.80** ±0.10	4.15 ±0.04	3.75** ±0.07

** P<0.01, *P<0.05, NS - Not significant, All values are Mean ± S.D.

enzyme synthesis explains the progressive loss of cation transport to a certain extent (Mathew 1998).

Although in the present study Na-K-ATPase and Ca-ATPase activity were not separately studied, it is reported that Ca-ATPase is considerably less sensitive to selenite than Na-K-ATPase (Hightower and Mc Cready 1991). An alternative explanation for calcium elevation includes impaired Na/Ca exchange process, or increased membrane permeability. Hightower and Mc Cready (1991) observations were based on the fact that the exposure of lenses to selenite caused both a voltage depolarisation, and an increased rate of chloride uptake–an indication that membrane integrity has been compromised. In the absence of evidence for calcium channels in the lens, it is assumed that calcium entry occurs by way of the many non-selective ion channels (Rae 1985), whose normal membrane permeability probably guard by the membrane SH groups.

The cation transport system in a cell is critical to main osmotic equilibrium, which, if disturbed, leads to osmotic swelling. Osmotic swelling contributes to cataract formation, possibly through physiological forces exerted on stretched membranes (Kinoshita 1974). A possible explanation of calcium ion accumulation is osmotic stress, due to an increased sodium ion concentration in the lens. Cation imbalances associated with impaired Na transport are historically associated with osmotic stress and lens hydration. Water is drawn into the lens fibre to maintain osmotic equilibrium, and an increase in electrolytes accompanies the water (Kinoshita 1964). It is proposed that the osmotic influxes of aqueous fluid include calcium (Hightower and Mc Cready 1989). Exposure to hyperosmotic media led to membrane leakness and opacity development (Jacob and Duncan 1980). These facts point at the possibility of osmotic stress-induced calcium accumulation.

Other supportive factors contributing to the altered cation transport is by inhibition of Na-K-ATPase activity by high levels of calcium (Hightower and Hind 1982). Additional contribution to the decline in transport includes decreased ATP availability, which was found decreasing in selenite injected rat pups (Hess et al 1983).

Magnesium is present in the lens as a major inorganic element, but little is known about the regulatory process of its level in the cells. Magnesium is known to be involved in the process of phosphate and nucleotide metabolism. In the present study, a highly significant decrease was observed in advanced selenite cataract stages. The loss is associated with the cortical involvement of cataract formation. In amphibian and mammalian lenses, the concentration of magnesium is higher in the cortical than the nuclear regions (Baldwin and Bentely 1980; Mc Gahan et al 1983). Since a highly significant loss was observed in advanced stages, it is due to an increased leakage to the aqueous and vitreous humours because of the altered membrane permeability (Mathew 1998).

During early selenite cataractous stage, an increased epithelial cell proliferation was noted (Mathew 1998). It is therefore possible that this increase in cell population results in the increase of number of active ATPase sites which would contribute towards maintaining total ATPase level comparable to that found in the controls. This is the reason for an insignificant decline of ATPase during early stages after selenite administration. But by the advancement of selenite cataract, many of the epithelial cells loss their control over cell multiplication, and results in hyperplasia formation (Mathew 1998). This results in a decrease of active enzyme sites, due to a reduction in the number of viable cells containing enzymes as observed in galactose cataract (Unakar and Tsui 1980). The decline in the ATPases activity observed in the present study alters the ion homeostasis and retards the energy metabolism of the lens.

The decline in ATPase activity can also be due to the decline in important lens antioxidants like GSH and ascorbic acids, which were observed during selenite cataractogenesis (Mathew 1998). The defensive mechanism of the lens is weakened, and the critical SH groups are likely to be oxidised much more easily. It has been suggested that GSH act as a protective agent for SH groups of ATPases, and a loss in GSH lead to the disulphide formation, thus contributing to the inactivation of the enzyme through a conformational change (Giblin et al 1976).

From the increasing facts of evidence it is concluded that target of selenium toxicity is the ATPase enzyme, and the membrane SH groups of the epithelium. The inactivation of ATPases or the oxidation of membrane sulfhydryls alters the membrane permeability and this result in the accumulation of sodium and calcium Mathew 1998).

The observed increase in calcium and sodium with a decrease in potassium and magnesium, in the present study, revealed that the normal ion homeostasis is altered during selenite cataractogenesis after different low and high doses (Tables 1, 2 and 3). This denotes an inactivation of ATPases, which is considered as the enzyme controlling the membrane transport of ions. Similar activity decline in ATPases and alterations in ion transport was reported during selenite cataractogenesis (Bunce and Hess 1981; Shearer et al 1992; Hightower and Mc Cready 1991). An increase in lenticular calcium during selenite cataractogenesis due to oxidation of the membrane SH groups activated the calcium-dependent proteolytic enzymes–calpain, which causes the proteolysis of crystallins and insolubilization of proteins resulting in opacity (Shearer et al 1992). Thus the maintenance of the membrane ion homeostasis is found to be critical, and that the ATPase enzyme has a leading role to play in keeping the lens transparent.The increasing evidences suggest that an activity decline in ATPase observed in the present study, is one among the causes for the development of the selenite-induced cataractogenesis.

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