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ANTIOXIDANT ENZYMES AND PEROXIDATION IN GALACTOGENIC CATARACT

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Abstract : The antioxidant enzymes like catalase and superoxide dismutase (SOD) were studied in erythrocytes and lens at various stages of cataractogenesis in albino rats. The rate of peroxidation was measured by assessing the malondiadehyde (MDA) in lens and plasma. The insoluble and soluble protein fractions were measured in lens to study the protein crosslinkings in relation to the above said parameters. Cataract was induced in albino rats by feeding it with 30% galactose as part of the normal diet (w/w) for 30 days. The results show a decrease of SOD and catalase with concomitant increase of MDA and insoluble protein with the advancement of cataract.

Key words : peroxidation free radicals H_2O_2 cataract

INTRODUCTION

Peroxidation has been associated with a number of specific manifestations related to cataract (1). The cataract formation through the polyol pathway is associated with free radical production and accelerates the damage of lens fibres (2). The lens is highly susceptible to superoxide anion or its derivatives and subjects exposed to hyperbasic oxygen develop cataract (3). Peroxide is catalysed by catalase and peroxides are synthesized through SOD. Lipids as structural components of lens fiber membranes are intimately associated with insoluble lens proteins. The increased insolubility of proteins with cataract formation may be due to derangements in the stereochemical arrangement between lipids and proteins in the membrane and the soluble proteins inside the fibers (4). The present works aims to study the SOD and catalase in relation to peroxidation and protein crosslinking during various stages of galactogenic cataracts in lens and erythrocytes.

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METHODS

Male albino rats (Sprague Dawley strain) weighing 80 ± 20 g of 28 days age were used for experimentation. Rats were divided into two, experimental and control groups. Control rats were provided with standard laboratory chow and experimental rats were provided with 30% galactose (w/w) as the part of normal chow for 30 days. Rats from both groups were sacrificed at 10 days interval to study the premature, mature and hypermature stages of cataract. The animals were killed by decapitation and blood was collected in a specimen bulb containing EDTA. The anticoagulated blood was centrifuged to separate plasma from packed cells. The eyes were dissected out and lens was separated in ice cold condition. Lens was sonicated and extract was prepared for carrying out the biochemical estimations. Heamolysate was prepared (5) from the packed cells and the enzymes like Catalase (6) and SOD (7) were estimated in haemolysate and lens extract. Peroxidation in lens and plasma were measured by estimating MDA (8). The water insoluble protein was separated (9) from the total protein and estimated (10).

RESULTS

The activity of catalase was found decreased in mature and hypermature stages of cataract in both tissues (Table I). However, the catalase activity of erythrocytes in premature cataractous group showed an increase on comparison with the controls. In the experimental group, a significant decrease in superoxide dismutase activity (Table I) was observed with the duration of experiment and also in comparison to the controls, both in lens and erythrocytes. It means activity of

TABLE I: SOD, Catalase, MDA in lens and erythrocytes.

Parameters	*Tissue*	*Group*	*Days*			*P<*		
			10	*20*	*30*	*10 vs 20*	*10 vs 30*	*20 vs 30*
SOD (IU/g Hb or protein)	Lens	Control	10.38±0.69	10.63±0.78	10.60±0.68	NS	NS	NS
		Experimental	8.48±0.76**	5.73±0.70**	4.60±1.21**	0.01	0.01	0.05
	RBC	Control	7.44±0.50	7.49±0.46	7.41±0.72	NS	NS	NS
		Experimental	5.37±0.69**	4.17±0.46**	3.08±0.72**	0.05	0.05	0.05
Catalase x 104 (IU/g Hb or protein)	Lens	Control	10.32±0.42	10.49±0.83	10.47±0.72	NS	NS	NS
		Experimental	10.41±0.55	6.55±0.74**	3.84±0.62**	0.01	0.01	0.01
	RBC	Control	17.45±0.52	17.24±0.55	17.48±0.55	NS	NS	NS
		Experimental	19.45±1.08**	12.82±0.42**	12.31±0.59**	0.01	0.01	NS
MDA (nmol/g protein or dl plasma)	Lens	Control	0.55±0.18	0.59±0.19	0.60±0.20	NS	NS	NS
		Experimental	0.90±0.37*	1.56±0.22**	2.98±0.01**	0.01	0.01	0.01
	Plasma	Control	0.93±0.28	0.99±0.06	0.93±0.24	NS	NS	NS
		Experimental	0.96±0.15	0.97±0.10	1.74±0.11**	NS	0.01	0.01

**P<0.01, *P<0.05, NS – Non significant

TABLE II: Lens proteins in normal and cataractous rats.

Parameters	*Tissue*	*Days*			*P<*		
		10	*20*	*30*	*10 vs 20*	*10 vs 30*	*20 vs 30*
Total protein (mg/g wet wt.)	Control	108.40±2.57	107.76±2.31	108.15±2.87	NS	NS	NS
	Experimental	96.76±8.02**	87.39±6.93**	77.36±2.52**	0.01	0.01	0.01
Water soluble protein (mg/g wet wt.)	Control	92.82±2.98	93.65±2.75	93.70±3.79	NS	NS	NS
	Experimental	67.47±3.59**	41.21±0.66**	28.78±3.36**	0.01	0.01	0.01
Water insoluble (mg/g wet wt.)	Control	15.59±4.06	14.10±0.93	14.38±4.46	NS	NS	NS
	Experimental	29.29±1.58**	36.18±0.66**	48.58±4.09**	0.01	0.01	0.01
Insoluble protein/ total protein	Control	0.14	0.13	0.13	–	–	–
	Experimental	0.30	0.41	0.63	–	–	–

**P<0.01, NS – Non significant

SOD decreases proportionately to the maturity of cataract.

The MDA showed an increase in the experimental groups in comparison to the control groups and also with the duration of experiment. The results indicate that the peroxidation plays an important role in galactose induced cataractogenesis. At the same time the plasma concentration of MDA was found significantly increased, only after 30 days of experimentation. The results indicate that the MDA in plasma was increased only after the hyper-maturation of cataract (Table I). The total protein as well as water soluble protein in lens of experimental group was decreased significantly along the maturity of cataract as well as in comparison to controls (Table II). But at the same time the water insoluble fraction found increased. The increase is consistent with the advancement of cataract.

DISCUSSION

The enzymes SOD and catalase protects the lens from highly toxic peroxides and from various kinds of oxygen radicals (11). SOD removes superoxide radicals by catalysing its dismutation to O_2 and H_2O_2 and catalase removes the resultant H_2O_2. The decreased activity of SOD in the present study might be due to the retroinhibition of H_2O_2, which was produced excessively in the lens. This conclusion gains support from our previous observations (12). The activity of catalase in the erythrocytes, at the initial stage might be due to the initial accumulation of peroxides in the erythrocytes. Later these accumulation leads to physio-chemical changes resulting in enzyme deactivation as observed in later stages. The inhibition of SOD may lead to the production of highly reactive oxidant, the hydroxyl radical (3). Superoxide free radicals (O_2^-) are generated during biological oxidations. The O_2^- and its derivatives like H_2O_2, singlet oxygen ($^1O_2$) and OH could be the primary source of oxidative damage in the cell (13). It has been proposed that the free radicals or factors possessing the properties of free radicals may be the

initiating agents in cataractogenesis (14). When the enzymatic defence against the toxicity of O_2^- being impaired, H_2O_2 and O_2^- could further react possibly through the Haber-Weiss reaction producing most potent oxidant, the hydroxyl radical.

MDA is an important indicator in the determination of degree of lens protein peroxidation. MDA was found to be increased with the advancement of cataract and is an evidence for peroxidation. There are two sources of peroxide formation in lenses viz., lenticular generation of reactive species of oxygen and the penetration of peroxides across the ciliary body into the aqueous humor from other tissue sites (15). The increase in peroxides with the maturation of cataract clearly explains the failure of scavenger system in lens and the involvement of MDA in cataractogenesis. In the initial phases of the experiment, the plasma MDA remained the same as that of the controls while it was found significantly increased in the lens. This confirms that the generation of peroxides had occurred inside the lens itself. At the final stage the increase of MDA in plasma may also contribute for the significant increase of MDA in lens. Galactose as well as other monosaccharides can auto-oxidize under physiological conditions, forming dicarbonyl compounds and hydrogen peroxide, both of which are potentially toxic (16). During the periods of elevated galactose levels, the auto-oxidation of galactose and concomitant increase in potentially toxic products could exceed detoxification capacity of lens (17). Radicals like O_2^-, H_2O_2 and the OH^- can react with nearby substrates and cause covalent modifications (18). Peroxides thus formed may alter membrane lipids and lens proteins which lead to cataractogenesis.

Under normal conditions, reduced glutathione may bind to the cysteine residues of lens proteins and protect the formation of disulphide bonds between cysteine residues of proteins. However, under abnormal conditions, the loss or reduction of GSH makes its thiol unavailable for protein protection. The unmasked sulphydryl groups of proteins readily form disulphide bonds between them resulting in protein aggregation or become insoluble (19). The amount of insoluble proteins increased in lens as the cataract advances. This may trigger a cascade of events, starting with enzyme deactivation, alteration of protein confirmation, protein-protein aggregation and eventually opacification. These huge protein species are believed to act as scatter points of light and causes the loss of lens transparency.

The failure of the antioxidant enzymes like catalase and SOD cause an increase in the peroxidation and generation of free radicals eventually leads to protein aggregation. The aggregation of protein and production of high molecular weight protein in ocular tissue alters the Donnan equilibrium. The ionic imbalance causes the inflow of water and subsequent swelling and destruction of the cell.

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