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Comparative Study of Enzymes in Normal and Cataractous Human Lens

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ABSTRACT:
Cataract is referred as opacity of lens due to multi factorial etiology. All the constituents of the protecting glutathione system including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transterase, g- glutamyl transpetidase were studied the mean value of GR activity is decreases under cataractous condition by 50.1%. Similarly, the GST activity and the GTP activity is also decrease under cataractous condition. It appears that the glutathione reductase system could still be functioning in the cataractous lens. The absence of oxidized glutathione in cataractous lenses would support this conclusion.

Key words: *Cataract, Glutathione Reductase (GR), Glutathione-s-Transterase (GST),* r-Glutamyl transpeptidase (GTP).

1. INTRODUCTION

Cataract is a major cause of blindness worldwide. The oxidation and reduction reactions in the lense play very important biochemical changes leading to opacification of lense in the eye causes cataract [1]. A single primary cause of cataract most likely does not exist. Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours [2]. Several risk factors listed in an elegant summary of the epidemiology of cataract are as follows.

1. Ionizing radiation
2. Radio frequency and micro radiation
3. Toxic drugs and Chemicals
4. UV-A (Ultra-violet-light long wavelength UV)
5. Diabetes, Blood pressure
6. Family history
7. Biochemical agents.

Lens is located between anterior aqueous and posterior vitreous humor. Being a vascular in nature, the lens contains no blood vessels, lymphatic, nerves or connective tissues.
The lens is a semi-solid, elastic, elliptical, refractive biconvex, a vascular highly organized cellular organ with smooth and shiny surface [3]. The lens proteins contribute 35% of the wet weight of the lens, and crystalline account for 80-90% of the soluble proteins of the lens.

Like other tissues, the lens contains high concentrations of glutathione. Oxidized glutathione produced by any of these mechanisms is reduced under the action of glutathione reductase; i.e.

G-S-S-G + NAD (P) H_2 → 2GSH + NAD(P)

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GLUTATHIONE REDUCTASE

Lens glutathione reductase is also capable of clearing mixed disulphide of glutathione and lens proteins. This provides the lens with a possible additional route for the regeneration of protein sulfhydryl. However, cataractous lenses contain substantial amount of mixed disulphide despite the presence of NAD (P) H and active glutathione reductase. Therefore it seems unlikely that glutathione reductase clears the mixed disulphide under physiological conditions. The reaction involving glutathione are summarized in figure-1. All the constituents of the protecting glutathione system including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transterase, g- Glutamyl transpetidase are studied.

2. MATERIALS AND METHODS

Fresh lenses were collected from hospitals and stored at very low temperature. Lenses were weighed and processed for enzymatic studies within few hours under cold condition. Following methods were used to determine enzymatic activities.

Assay for Glutathione reductase (GR) activity (EC No. 1.6.4.2)
GR activity was estimated by the method of Carlberg and Mannervij [4].

Assay for Glutathione S-Transferase (GST) activity (EC NO. 2.5.18)
The GST activity was assayed by Wormhole et al.[5] method.

Assay for r-Glutamyl transpeptidase (GTP) activity (E.C. No. 2.3.2.2.)

The GTP activity was assayed by the method of Tate and Meister [6].

The activity was expressed as units/hr/g fresh lens, where a unit of GTP is equal to the u moles of p-nitroaniline released per minute. Lenses were classified by Chylack L.T.Jr's classification. 86 [Chylack L.T.Jr's Arch. Opthalmol. (1993), 111, 831]

3. RESULTS AND DISCUSSION

Glutathione Reductase (GR), Glutathione-S-Transferees (GST), r-Glutamyl Transpetidase (GTP) and different age 98 groups of normal human lenses are matched as per table-1. The minimum enzymatic activity was 99 obtained in the last age group i.e. 81-90 years, for all the three enzymes. There is no significant 100 difference in enzyme activities in other age groups i.e. 41-50, 51-60, 61-70, 71-80 years.

3.1. Glutathione reductase (GR)

The function of high level of GSH in the normal lens is to protect the protein-SH groups by preventing their oxidation and reversing any oxidation which had taken place. The GSH reversing any oxidation which had taken place. The GSH can act as a scavenger for any free radicals generated and is oxidized to a disulfide in the process. Lens glutathione reeducates is also capable of cleaving mixed disulphide of glutathione and lens proteins [1]. This oxidized GSH is reduced by Glutathione reductase as below. Reported results show activity of this enzyme reduces during aging and cataractogenesis. However, cataractous lenses contain substantial amount of GR and mixed disulphides. Other groups of scientist have also reported decrease activity of GR [7]. The fall in GR activity would result in decrease in GSH concentration, since, the system loses its capacity to regenerate GSH from G-S-S-G. However, increased translocation of GSH due to altered membrane permeability and other mechanisms responsible for the decrease in the content of GSH in cataractous lenses may not be excluded. i.e. decreased r-Glutamyl cysteine synthetase activity [8].

Another mechanism that causes decrease in GSH level is activity of HMP shunt which is the source of production of NADPH is affected in galactose and alloxan [9,10] treated lenses. The decrease in GR activity will also affect the proteins, since the fall in GSH levels would result in disturbances in the maintenance of protein-SH groups in reduced form giving rise to protein -protein disulfide bonds or protein - GSH disulfide bonding leading to aggregation of these proteins.

An explanation for fall in GR activity would be the inhibitory effect of oxidants on the activity of reducing enzymes [11]. For the same reason GR is an-SH dependent enzyme. Since photo or chemical oxidation of specific amino acids (i.e. Tryptophan) can react with proteins and GSH SH groups. It may be postulated that the oxidative loss of GR activity is due to tying – up 1of essential –SH groups in the enzyme [11]. On the basis of the above preliminary results, it appears that the glutathione reductase system could still be functioning in the cataractous lens. The absence of oxidized glutathione in cataractous lenses[12] would support this conclusion. Nevertheless protein disulphide bonds still accumulate slowly. Perhaps, this indicates that the GR system cannot quite cope with the rate of oxidation in the cataractous lens. Decreases activity of GR, cataractous groups evokes changes in the lens epithelium as GR provides reducing capacity for the formation of DNA [13]. Thus from the above inferences, it may be speculated that the dimination of GR activity could affect two major constituent or the lens-proteins and GSH, leading to the accumulation of GSSG, and H_2O_2 which is toxic to the lens.

3.2. Glutathione- S-transferase (GST)

One of the most important enzymes which is associated with GSH degradation is GST. The results suggest a significant loss of the activity of such enzyme may be concerned with GSH 1degradation in the more advanced stages of all types of cataract studied. Besides maintaining a reduced condition, scavenging role of GSH has been proposed in various tissues. GST, a family of proteins having multiple detoxification effects have been shown to enzymatically catalyze the conjugation of hydrophobic compounds bearing an electrophonic groups with endogenous nucleophile GSH in the first step of mercapturic acid biosynthesis [14]. It was also observed that the activity of GST decreased with advancing age and severely decreased under cataractous condition i.e. 70% compared to normal human lens. One of them has reported 73% reduced activity of GST in the brown dense cataracts [7].

The loss in activity of GST should result in accumulation of GSM during cataractogenesis. However, the opposite occurs: the GSH is decreased compared to their respective controls i.e. normal lenses. Thus, a decreased GH synthesis or increased lens GSH leak out may account for the marked loss during cataract formation. With respect to the xenobiotics' removal and detoxifying function of GST, the activity of the enzyme in cataractous lenses is more than sufficient to remove any potential cataractogenic compound via mercapturic acid pathway. Among the enzymes of

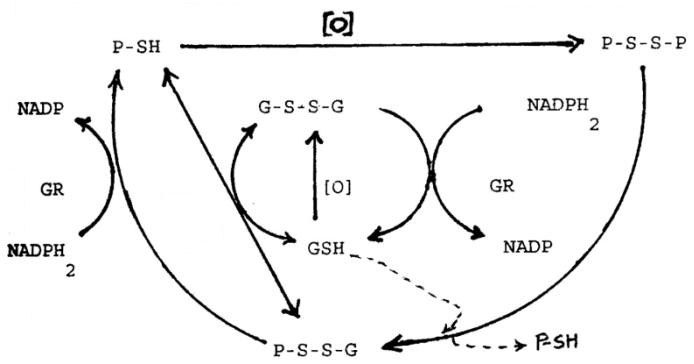

Figure 3. The glutathione protective system

Table 1. GR, GST and GTP in normal human lens in different age groups.

Age (years)	GR (n moles/ min/mg)	GST (n moles/ min/mg)	GTP (n moles/min/ mg)
41-50(5)	1.620±0.091(5)	2.1703±0.083(3)	9.9121±0.11(6)
51-60(5)	1.339±0.071(4)	1.6398±0.064(4)	11.1342±0.09(4)
61-70(4)	1.521±0.084(5)	1.7906±0.069(4)	11.0781±0.14(4)
71-80(5)	1.519±0.047(4)	1.7781±0.058(5)	8.6237±0.18(3)
81-90(4)	1.316±0.079(4)	1.5240±0.071(5)	7.2311±0.09(5)

All values are expressed as mean ± S.E.
Numbers in the parenthesis are sample sizes.
P-value ∗ < 0.01

Table 2. GR, GST and GTP activities in normal and cataractous human lenses.

Type	GR (n moles/min/mg)	GST (n moles/ min/mg)	GTP (n moles/min/mg)
Normal 54±11Yrs	1.463 ± 0.079 (n = 22)	1.780±0.069 (N=20)	9.595±0.094 (N=22)
Cataractous 58±14yrs	0.730±0.062 (n=42)	0.545±0.342 (N=45)	3.70±0.216 (N=39)

All values are expressed as mean ± S.E.
Numbers in the parenthesis are sample sizes (n).
For all p-value < 0.01

the mercapturic pathway in lens, the activities of the GST are significantly higher than GTP.This enzyme was interjected between glutathione synthetase and r-GTP in the glutathione metabolizing cycle, because of wide range ofsubstrate specificity of GST compared to r-GTP [5].

3.3. R- Glutamyl transpeptidase (GTP)

r-GTP is an important enzyme of r-Glutamyl cycle and initiates GSH degradation. It has there catalytic activity : (1) Transpeptidation-r-Glutamyl moiety is transferred to an acceptor, (2) Auto Transpeptidation - r-Glutamyl moiety is transferred to GSH to form r -Glutamyl-GSH (3) Hydrolysis - r-glutamyl moiety is transferred to water. The various substrates for the same enzymes are glutathione, oxidized glutathione, s-substituted glutathione and other r-Glutamyl compounds [5].

Since GSH is entirely degraded within the lens [15], the r-Glutamyl cycle seems to play an important role in the lens. GTP reacts very effectively with GST amongst all the enzymes involved in the GSH cycle. The activity of GTP is very low compared to GR and GST as reported here. There is no significant relationship between age and activity of GTP. The degradation of GSH by GTP is thought to be coupled with transport of amino acids across the membrane by the same enzyme. This mechanism is highly effective in the lens, since it has a rapid turnover of GSH and is

able to transport amino acids in to the tissue [16]. Any change in such mechanism may alter the GTP activity in the lens). The GTP activity level was found to remain steady in the initial stages of post-natal development and then there was a sudden surge in the activity in the adult lenses.

5. CONCLUSION

The term cataract implies opacification of the lens, this being almost without exception the only pathological change which the lens can undergo. The adjective "senile" has become attached to the most common of all forms of cataract, about the precise etiology of which nothing is known because cataract is a multifactorial process in which many intrinsic and extrinsic factors act cumulatively. To prevent or retard (delay) the cataract, risk factors for lens damage should be minimized and biologic defense system should be maintained at optimal strengths. The GR, GST and GTP activities in normal human lenses are 1.463 in n moles/min/mg, 1.78 n moles/min/mg and 9.moles/min/mg where as in cataractous lenses, these are 0.73 n moles/min/mg, 0545 n moles/min/mg and 3.70 n moles/min/mg respectively.

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