Influence of galactose cataract on erythrocytic and lenticular glutathione metabolism in albino rats

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Context: Glutathione depletion has been postulated to be the prime reason for galactose cataract. The current research seeks the prospect of targeting erythrocytes to pursue the lens metabolism by studying the glutathione system. **Aims:** To study the activity of the glutathione-linked scavenger enzyme system in the erythrocyte and lens of rats with cataract. **Materials and Methods:** Experiments were conducted in 36 male albino rats weighing 80 ± 20 g of 28 days of age. The rats were divided into two major groups, viz. experimental and control. Six rats in each group were sacrificed every 10 days, for 30 days. Cataract was induced in the experimental group by feeding the rats 30% galactose (w/w). The involvement of reduced glutathione (GSH) and the linked enzymes was studied in the erythrocytes and lens of cataractous as well as control rats. **Statistical Analysis:** Parametric tests like one-way ANOVA and Student's 't' test were used for comparison. Correlation linear plot was used to compare the erythrocyte and lens metabolism. **Results:** The concentration of GSH and the activity of linked enzymes were found decreased with the progression of cataract, and also in comparison to the control. The same linear fashion was also observed in the erythrocytes. **Conclusion:** Depletion of GSH was the prime factor for initiating galactose cataract in the rat model. This depletion may in turn result in enzyme inactivation leading to cross-linking of protein and glycation. The correlation analysis specifies that the biochemical mechanism in the erythrocytes and lens is similar in the rat model.

Key words: Cataract, erythrocytes, galactose, glutathione, lens

Cataract is the loss of transparency of the lens which develops as a result of altered physical and chemical processes in its colloids. Experimental cataract has helped a lot to explicate the underlying unsolved biochemical mechanism of cataract formation. Extensive research revealed a great deal about the underlying mechanism responsible for the development of opacities. The lens contains an unusually high concentration of glutathione. Most of the lens glutathione is in the reduced form (GSH) i.e., only 6.8% of total lens glutathione is in the oxidized form (GSSG).[1] Glutathione plays an important role in the metabolism of the lens, particularly in the maintenance of transparency of the lens tissue.[2,3] GSH is enzymatically synthesized in the lens and also plays the role of a scavenger for free radicals produced by oxidative stress via the oxidation-reduction system.[4,5] During all the experimental cataracts, the concentration of these short peptides or GSH/GSSG ratio was found to be decreased.[1,6-8] Depending on the magnitude and persistence of the cataractogenic insult, the leakage of glutathione may continue or stop. The aim of the present work was to study the involvement of erythrocytic and lenticular glutathione and its linked enzymes in relation to galactogenic cataract.

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Materials and Methods

Experiments were conducted in 36 male albino rats (*Rattus norvigicus*) of Sprague Dawley strain after obtaining ethical committee approval. Male rats weighing 80 ± 20 g of 28 days of age were used for experiments and were maintained as per ethical committee guidelines of the Institution. Rats were divided into two major groups, viz. experimental and control, each of which was again divided into three subgroups. All the subgroups contained six rats (n = 6) and were assigned as 10 days, 20 days and 30 days. At the end of every 10 days one group (n = 6) from the experimental and control was sacrificed as designated and this was continued up to 30 days. Cataract was induced in the experimental group by feeding rats with a 30% galactose diet along with the normal diet. The formation of cataract in the lens was confirmed by the visible ophthalmologic examination. Control group was fed with standard laboratory chow of Amrut Feeds India Ltd. (Hindustan Lever). Rats were killed by decapitation and blood was collected in anti-coagulated vials (n = 6, for each group). The eyeballs (n = 12, two from each rat) were dissected out immediately and the lenses were encapsulated and kept at ice cold condition. The anti-coagulated blood was centrifuged at 1500 rpm for 5 min for plasma separation. The packed cells were washed with isotonic saline (physiological saline) and centrifuged at 3400 rpm for 30 sec. This was repeated three to four times and the packed cells were kept at -4°C to freeze. Hemolysate was prepared by the continuous freezing and thawing method as described by Beutler.[9] The eye lens obtained by encapsulation was sonicated in 1*M* Tris HCL buffer at ice cold condition. The 10% lens extract was prepared for carrying out the biochemical estimations. The activities of glutathione reductase (GR), glutathione peroxidase (GSHPx), glutathione-s-transferase (GsT) and the concentration

of GSH were estimated using the standard methods[9] in lens and erythrocytes. The activities were expressed in terms of hemoglobin (Hb)[9] for erythrocytes and protein[10] for lens. The normal distribution pattern of the data was confirmed for conducting parametric statistical tests. The experimental and control groups were compared by students 't' test and inter-group variations were analyzed by one-way ANOVA. Curve expert software Version 1.34 was used for correlation between tissues by linear plot.

Results

GSH level and the glutathione-linked enzymes like GR, GSHPx and GsT showed a significant reduction in comparison to the control groups in lens (N= 12) [Table 1] and erythrocytes (N=6) [Table 2]. The decrease became more prominent with the progression of the cataract. It is clear from the table that the enzymes were getting deactivated with the maturation of the galactose cataract in the rat model. The depletion of the GSH corresponded with the inactivation of these enzymes. The comparison of various enzymatic trends [Fig. 1] between the lens and erythrocyte of experimental rats indicated the same pattern of metabolic changes in the tissues. This is also supported by the correlation analysis [Fig. 2]. It shows a positive correlation between the two tissues (r = 0.87) as far as glutathione metabolism is concerned.

Discussion

The lens and erythrocytes contain an unusually high concentration of GSH and the concentration of this peptide decreases in almost all types of cataract.[1,6,11,12] GSH is a coenzyme for several enzymatic reactions in addition to its role in the oxidation-reduction reaction.[13] There are many similarities between erythrocytes and lens cells. Both are filled with a highly concentrated solution of specialized proteins. Precipitation of these proteins in red cells produces hemolytic anemia whereas the precipitation of lens proteins leads to cataract.[11] GSH is enzymatically synthesized in the lens and acts as a scavenger for free radicals produced by oxidative stress.[1] However, the decrease of GSH in the experimental group may be either due to the decreased synthesis of GSH or its increased oxidation to GSSG and subsequent leakage. The loss of GSH is brought about by its oxidation. The oxidized form apparently can leave the lens more rapidly than GSH, possibly due to the increased permeability of lens membranes.[14]

The enzymes GSH-Px, GR and G6PD take part in the defense against the reactive species of oxygen which are responsible for increased oxidative stress in erythrocytic and ocular tissues.[4-6,15] GR is an important enzyme in maintaining GSH as well as protein sulfhydryls in reduced state. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) required for the glutathione reductase reaction is regenerated

Table 1: Glutathione-linked enzymes in lens

Enzyme	Group	10 days	20 days	30 days	Fischer "F" value	Level of significance
GSH (µmole /g protein)	Control	14.81 ± 1.81	15.57 ± 1.80	16.34 ± 1.70	2.24	NS
	Experi-mental	8.33** ± 1.04	6.49** ± 0.69	2.60** ± 0.70	147.43	P<0.01
GsT (IU/g protein)	Control	6.33 ± 1.22	6.16 ± 1.07	6.62 ± 1.84	0.32	NS
	Experi-mental	5.29* ± 0.73	3.94** ± 0.95	2.04** ± 0.52	56.26	P<0.01
GR (IU/g protein)	Control	6.91 ± 0.42	6.73 ± 0.56	6.97 ± 0.44	0.82	NS
	Experi-mental	3.74** ± 0.46	2.44** ± 0.63	1.52 ± 0.39	58.88	P<0.01
GSH-Px (IU/g protein)	Control	53.38 ± 2.09	53.41 ± 3.38	51.06 ± 2.67	2.88	NS
	Experi-mental	35.52** ± 4.05	22.01** ± 2.56	5.56** ± 1.72	256.51	P<0.01

**$P<0.01$ (t = 3.169), *$P<0.05$ (t= 2.228) students "t" comparison with control, GSH: Reduced glutathione, GsT: Glutathione s-transferase, GR: Glutathione reductase, GSH-Px: Glutathione peroxidase, P: level of significance, NS: Non-significant

Table 2: Glutathione-linked enzymes in erythrocytes

Enzyme	Group	10 days	20 days	30 days	Fischer "F" value	Level of significance
GSH (µmole /g Hb)	Control	2.67 ± 0.33	2.53 ± 0.27	2.65 ± 0.39	0.309	NS
	Experi-mental	1.75** ± 0.15	0.95** ± 0.11	0.49** ± 0.15	128.154	P< 0.01
GsT (IU/g Hb)	Control	5.06 ± 0.49	5.40 ± 0.50	5.10 ± 0.53	0.806	NS
	Experi-mental	5.27 ± 0.30	5.14 ± 0.12	3.95** ± 0.67	17.217	P<0.01
GR (IU/g Hb)	Control	2.85 ± 0.27	2.86 ± 0.25	2.84 ± 0.15	0.011	NS
	Experi-mental	2.79 ± 0.30	2.01 ± 0.14	1.07** ± 0.14	103.337	P<0.01
GSH-Px (IU/g Hb)	Control	55.57 ± 4.30	54.01 ± 3.45	58.84 ± 3.56	2.539	NS
	Experi-mental	65.01** ± 3.91	45.91** ± 2.44	34.45** ± 4.56	102.061	P<0.01

**Students "t" comparison with control, t value above 4.604 ($P<0.01$), *Students "t" comparison with control, t value above 2.667 ($P<0.05$), GSH: Reduced glutathione, GsT: Glutathione s-transferase, GR: Glutathione reductase, GSH- Px: Glutathione peroxidase, P: level of significance, NS: Non-significant
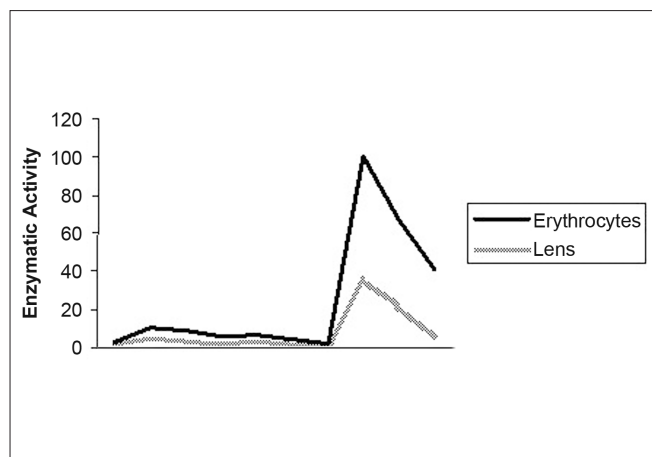
Figure 1: Enzymatic activity in lens and erythrocytes

Figure 2: Linear plot showing correlation between lens and erythrocytes

from nicotinamide adenine dinucleotide phosphate (NADP) by glucose 6 phosphate dehydrogenase through the hexose monophosphate shunt,[14] which constitutes the glutathione redox cycle.[16] This requires elevated levels of NADPH to reduce the glutathione disulphide produced during hydro-peroxide metabolism. Therefore, a depletion of NADPH release to the GSH redox cycle might increase oxidative stress favoring the development of complications. Decreased GSH levels may also be due to the over-activity of the polyol pathway, which decreases the supply of NADPH to the GSH redox cycle.[16] Gonzalez *et al.*,[17] reported that aldose reductase has tenfold lower Km value for NADPH than GR. Hence, the NADPH utilization by aldose reductase will be more than that of GR. Due to this reason regeneration of GSH from GSSG may be severely affected. However, it has to be accentuated that such a shunting through the polyol pathway may occur in the sugar-induced cataracts only.

GSH-Px converts GSH to its oxidized form, GSSG and thereby removes toxic peroxides. The decrease in the activity of GSH-Px in lenses of the experimental group accounts for decreased availability of GSH which in turn reduces the removal of H_2O_2. It was shown that the enzyme is highly specific for GSH, but operates on a variety of peroxides, besides hydrogen peroxide and thus it is concerned with the elimination of toxic peroxides.[18,19] GsT, the first enzyme in the mercapturic acid pathway, catalyses the nucleophilic addition of the thiol of GSH to electrophilic acceptors, such as organic peroxides, quinones, aryl and alkyl halides, olefins and sulphate esters. The level of glutathione and the activity of GsT are very high in the lens, and the mercapturic acid pathway plays an important role as a scavenging system in the lens.[20] In the present experiment, GsT was also found to be decreased along with GSH, with the progress of cataract, which explains the failure of scavenging systems in the lens and erythrocytes.

Advanced glycation end products (AGE) formed by the auto-oxidation of galactose can result in the protein cross-linkings in cataract.[4,21] Such type of cross-linkings may deactivate the enzymes under consideration leading to depletion of GSH. In short, the studies on the glutathione cycle in galactose-induced cataract showed a decrease in GSH content. This decrease
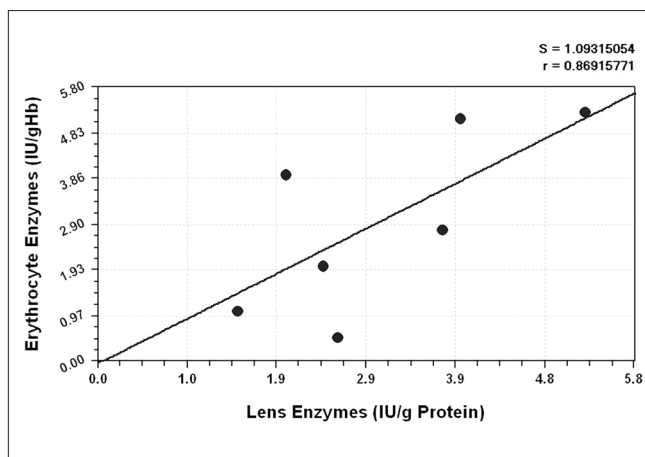
is in concordance with the decreased activity of reductase, transferase and peroxidase enzymes, which may be due to reduced supply of the NADPH to the glutathione cycle. The failure of regeneration of GSH and the resultant depletion of linked enzymes (GsT and GPx), leads to the precipitation of lens proteins leading to cataract. The same result can also be observed in the erythrocytes, which have the same morphology as the lens. However, based on the findings such changes can be generalized for galactose cataract in a rat model and further research is needed to trace this in other forms of cataract.

In conclusion, the depletion of GSH acts as the prime factor for initiating galactose cataract in rat model. This depletion may in turn result in enzyme inactivation leading to cross-linking of protein and glycation. The correlation analysis specified that the biochemical mechanism in erythrocytes and lens was similar in a rat model.

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