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GLUTATHIONE ASCORBIC ACID OXIDATIVE MECHANISM IN HUMAN CATARACTOUS LENSES

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Abstract. An attempt was made to modify and elaborate the microestimation methods for the determination of reduced glutathione and ascorbic acid in the blood, aqueous humour and lenses of human cataractous patients. The concentration of glutathione and ascorbic acid in different types of cataractous lenses were compared. It was concluded that glutathione when present in high concentration inhibits the oxidative mechanism.

It is generally known that tissue metabolism is first anaerobic by glycolytic pathway to form lactic acid and then gives carbon dioxide by process known as citric acid cycle. In the lens there is low oxygen consumption, therefore, citric acid cycle plays a minor role in carbohydrate metabolism and direct oxidative cycle is of great significance. TPN is the reduced coenzyme in Shunt mechanism and it should be reoxidized to provide energy to the lens. This reoxidation in the other tissue is effected by the chain of respiratory enzymes involving the flavoproteins and the cytochromes.

Heyningen and Pirie[1] reported an enzyme glutathione reductase in the lens in the presence of this enzyme, oxidized glutathione (GSSG) is reduced to glutathione (GSH) and the TPNH in turn is oxidized to TPN. Dische and his coworkers[2] reported that the lens capsule is capable of oxidizing glutathione. Kinoshita[3] observed that dehydroascorbic acid enhances the oxidation by increasing the availability of TPN in the system. The high concentration of glutathione and ascorbic acid in lens supports the possibility that glutathione–ascorbic acid oxidative mechanism exist, in the lens. Moreover, the lens is acellular and contains less mitochondria which is the seat for cytochrome system, the reoxidation of TPNH by cytochrome system is not very efficient in the lense. It has been shown that the glutathione reductase is found in the soluble fraction,[4] therefore, a soluble and diffusible enzyme system was proposed[3] to exist in the lens.

To establish the glutathione ascorbic acid oxidation mechanism playing a role in the lens metabolism, a number of experiments have been conducted. The present study was taken up to study this oxidative mechanism in cataractous lens, with special reference to diabetic cataracts.

Material and Methods

Source of Lenses, Aqueous Humour and Blood. Human cataractous lenses were removed at the time of operation in the Department of Ophthalmology, Jinnah Postgraduate Medical Centre, Karachi. The lenses were preserved in the lens preservative solution (glycerol+phosphate buffer, pH 7) and brought to the laboratory under deep-freeze conditions, soon after operation for biochemical analysis. Aqueous humour (about 0.3–0.4 ml) and the blood of all the patients operated were collected. The lenses were freshly weighed and homogenized in glass homogenizer containing 1 ml distilled water. Reduced glutathione (GSH) and reduced ascorbate were estimated in blood, aqueous humour and lens homogenate by the method described below.

Estimation of Glutathione (Grunert and Phillips Method). The reduced glutathione was estimated by the method described by Grunert and Phillips.[5] The principle involves the removal of proteins, and protein-free filtrate is treated with sodium nitroprusside and sodium cyanide–sodium carbonate mixture in the presence of sodium chloride. The colour developed is compared spectrophotometrically with standard treated similarly.

Reagents

1. Sodium cyanide (0.067 ml) in 1.5 ml sodium carbonate solution. Sodium carbonate (39.75 g) was dissolved in water and made to 250 ml then 0.821 g sodium cyanide was added and dissolved.
2. Sodium chloride solution (saturated).
3. Sodium nitropruside (2% w/v). The solution was prepared immediately before use and kept in an ice-bath.
4. Sodium tungstate (10% w/v).
5. Sulphuric acid (2/3N). 18.0 ml 36N sulphuric acid was added slowly to water and made to one litre. The normality was checked by titrating against standard alkali.
6. Reduced glutathione (Kochlight Laboratories) was used as standard. Reduced glutathione (100 mg) was dissolved in distilled water and made to 100 ml. The standard was kept in deepfreeze.

Method of Assay. Blood, aqueous humour or lens homogenate (0.1 ml) was added in centrifuge tube containing 0.7 ml distilled water. After complete hemolysis 0.1 ml 10% sodium tungstate and 0.1 ml 2/3N sulphuric acid were added and then centrifuged.

To 0.6 ml saturated sodium chloride 0.2 ml supernatent and 1 ml sodium nitroprusside were added. Then the optical density was read at 520 nm after addition of 0.1 ml sodium cyanide–sodium corbonate solution.

Reagent blank was prepared in the same manner except that 0.2 ml distilled water was used instead of supernatent. The glutathione concentration was calculated in mg %.

Estimation of Ascorbic Acid. Reduced ascorbic acid was estimated in the blood, aqueous humour and lens homogenate, by the method described by Owen and Izgo.[6] This method depends on the reduction of 2:6-dichlorophenolindophenol by ascorbic acid at a pH of about 4.0. The residual unreduced pink dye is measured at 520 nm on Spectronic '20' spectrophotometer. Only a very small fraction of or none of the vitamin C in plasma is dehydroascorbic acid so that this method gives the same results as the more time-consuming method depending on formation of 2:6-dinitrophenylhydrozone of dehydroascorbic acid after oxidation of vitamin C.

Reagents

1. Metaphosphroic acid (3% w/v aqueous solution). This was prepared fresh for each estimation and altered before use through a Whatman No. 42 filter paper.
2. Sodium citrate (10% w/v aqueous solution).
3. Sodium citrate (50% w/v aqueous solution).
4. 2:6-Dichlorophenolindophenol.

One B.D.H. tablet in water was dissolved and made up to 5 ml and altered.

5. Ascorbic Acid (100 mg/100 ml): This stock solution was dissolved to give standard solutions containing 0.25, 0.5. and 1.0 mg/100 ml. Stock and standards were prepared immediately before use.

Methods of Assay. Plasma and Aqueous Humour: Protein-free filtrates of human plasma (oxolated or heparinized) were prepared by adding 2 volume of plasma to 3 volume of 3% w/v metaphosporic acid.

Lens Homogenate. Two volumes of lens homogenate were added to 3 volumes of 6% metaphospric acid and then centrifuged. pH Measurement were done on pH meter.

The measurement of the indophenol reducing activity was conducted in the cuvette. Test solution (0.2 ml) was pipetted out following by 0.6 ml sodium citrate (concentration according to the final pH required) and 1.8 ml dye indophenol. After rapid mixing the optical density of the solution was measured with a Spectronic '20' spectrophotometer at 520 nm at 30 sec.

Results

In the present study an attempt was made to modify and elaborate the microestimation methods for the determination of reduced glutathione and reduced ascorbic acid in the blood, aqueous humour and the homogenates of different lenses. The table shows the summary of the reduced glutathione and reduced ascorbic acid concentration in the blood, aqueous humour and lens homogenates of different forms of cataractous lenses.

The relations between the milligram per cent concentration of the glutathione in blood, aqueous humour and different types of lenses is given in Table 1 and the milligram per cent concentration of ascorbic acid in blood, aqueous humour and the different types of lens homogenates is also given in Table 1.

The concentration of the reduced glutathione increases as the opacity of the lens increases. It is maximum in case of morgagnian or diabetic cataract. The rise in concentration level is accompanied by the rise in blood and aqueous humour (Table 1).

Milligram per cent concentration of ascorbic acid and (GSH) in blood and aqueous humour of normal persons studied are given in Table 2. This shows a gradual fall in the concentration with the appearances maturation of cataractogenesis. In the blood there is little decrease in the ascorbic acid concentration while there is an appreciable decrease in the ascorbic acid concentration of lens, specially in case of diabetic cataract or morgagnian cataract.

Discussion

Glutathione is tripeptide of glutamic acid, cystein and glycine, is present in blood but its biochemical function is not completely understood. It is believed that glutathione protects sulphydryl groups of various proteins. Its function in erythrocytes is to maintain the integrity of the cell by protecting the sulphydryl groups of haemoglobin, catalase and lipo-proteins of cell membranes. Somewhat similar mechanism of glutathione has been reported to exist here.

Glutathione which is synthesised in the lens, is probably actively transported out of the lens when it becomes oxidized.[7] Mach[8] found average levels of 40–70 μg/10 mg (1.3–2.3 μ-moles/g) less compared with that of the average values of 215 μg in a normal lens (7.0 moles/g). Consul and Nagal[9] reported a decrease from 350 mg/100 g to 3 μg(11.4–1.0μ-moles/g) in mature cataract. In the present study the glutathione (GSH) concentration in the lens is less than the normal in immature cataract. It gradually increases in the mature and hypermature cataracts and is highly elevated in morgagnian or diabetic cataract. These results are in contradiction to the finding of Dickison *et al.*[10] who found a tendency in 12 cataractuous lenses, for the content of glutathione and amino acids to be lower, the more advanced the cataract.

The ascorbic acid concentration in the immature cataract is normal. It decreases as the opacity increases. It is greatly lower in the morgagnian or the diabetic cataract. It has been reported that the ascorbic acid is lower in case of senile cataract.[9,11] The concentration of the reduced glutathione and ascorbic acid in the blood and aqueous humour in case of immature cataract is approximately normal. However, small

TABLE 1. GLUTATHIONE ASCORBIC ACID OXIDATIVE MECHANISM IN HUMAN CATRACTOGENIC LENSES RESULTS SHOWING THE CONCENTRATIONS OF REDUCED GLUTATHIONE AND REDUCED ASCORBIC ACID THE BLOOD AQUEOUS HUMOUR AND LENSES OF DIFFERENT CATARACT OF HUMAN PATIENTS.

Types of cataracts	Reduced glutathione (GSH) (mg%)			Reduced ascorbic acid (mg %)		
	Concn in blood	Concn in aquous humour	Concn in lenses	Concn in blood	Concn in aquous humour	Concn in lenses
Immature	18.00	17.00	4.00	3.00	18.80	33.50
Mature	23.20	21.00	9.00	2.00	14.00	28.00
Hypermature	29.00	27.00	12.00	1.80	10.00	20.00
Morgagnian	39.00	26.00	16.00	1.25	8.40	18.00

TABLE 2. THE CONCENTRATIONS OF REDUCED GLUTATHIONE AND REDUCED ASCORBIC ACID IN THE BLOOD AND AQUEOUS HUMOUR OF NORMAL PERSONS.

Reduced glutathione (mg %)		Reduced ascorbic acid (mg %)	
Concn in blood	Concn in aqueous humour	Concn in blood	Concn in aqueous humour
18.4	17.0	1.37	12.0
19.5	17.5	1.10	10.5
18.5	17.0	1.15	17.0

fluctuations in the glutathione and ascorbic acid level in the blood bring about large changes in the aqueous humour and also in the lens, and this would lend to obscure lens changes due to cataract. Somogyi[12] was the first to suggest that the glutathione was chiefly responsible for the reduction yielded by blood filtrate after fermentation with yeast. Benedict and Newton (cited by Fashena[13]) isolated glutathione from sheep blood and assumed that it was responsible for a large proportion of the non-sugar reduction yielded by blood. As far as the lenses are concerned the latest report of Heymingen[14] suggested that the accumulation of ascorbic acid in the lens of napthalene fed rabbit is due to the penetration of dehydroascorbic acid formed in this way and reduced in the lens to ascorbic acid by glutathione. This idea is strengthened by the finding that dehydroascorbic acid itself readily penetrates the lens *in vitro*.

In the light of present studies and the views presented in the recent study of Heyningen[14] it is easy to conclude that the glutathione when present in high concentration inhibits oxidative mechanism, moreover, causes inhibition of soluble proteins leading to cataract complications. Similarly the lower concentration of the ascorbic acid in the complex cataract specially in morgagnian cataract retards the glutathione (GSH) and ascorbic acid oxidative mechanism. Thus it is known for certain in the light of present study that this oxidative mechanism plays an important role in the lens metabolism. In order to establish the exact role played by glutathione, further biochemical studied are in progress in our Department.

Summary

The present study was taken up to study the oxidative mechanism in cataractous lenses. Blood, aqueous humour and cataracts removed at operation were collected for biochemical analysis. Microestimation methods for the determination of reduced glutathione (GSH) and ascorbic acid were modified and elaborated. The relation between the milligrams per cent concentration of glutathione and ascorbic acid in different types of cataracts (classified on the basis of colour and opacities) were determined.

The results were compared with the normal blood and aqueous humour glutathione (GSH) concentration in immature cataract and was found to be less than in the normal. It increases in the mature and hypermature cataracts and highly elevated in morgagnian cataracts. The ascorbic acid concentration in immature cataract was comparable to normal ones. It decreases as the opacity increases. Thus it was concluded that the glutathione when present in high concentration, inhibits the oxidative mechanism.

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