EFFECT OF KETOACIDS ON H₂O₂ INDUCED CATARACT

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ABSTRACT

Cataract is one of the leading causes of visual disability often leading to blindness in the elderly population. One of the causes is oxidation of proteins present in lens, by hydrogen peroxide (H₂O₂). In the present study 100 goat lenses were analyzed to determine the protective efficacy of ketoacids, against the oxidative insult by H₂O₂. The ketoacids used were (pyruvate, alpha ketoglutarate and oxaloacetate), that are constantly produced endogenously. The lenses were incubated as control and experimental groups in TC-199 media for 72 hrs. H₂O₂ concentration of 10mM was used to induce cataract. The biochemical parameters measured were levels of malondialdehyde (MDA), a lipid peroxidation product and activity of glutathione peroxidase (G-Px), an enzymatic antioxidant. The results showed a significant increase in the levels of MDA and significant decrease in the activity of G-Px in the cataractous lenses as compared to control. After addition of ketoacids (pyruvate (10mM), alpha ketoglutarate (20mM) and oxaloacetate (20mM)) separately, the levels of MDA decreased significantly and the activity of G-Px increased significantly. The results suggest that the ketoacids can be very promising antioxidants for the treatment of cataract. They may also be useful in treating other disabilities related to acute and chronic oxidative stress.

KEY WORDS

cataract; oxidative stress; antioxidant; ketoacids

INTRODUCTION

Cataracts are formed as a result of natural aging processes, injury, metabolic changes, drugs, toxic chemicals or various types of radiation. Most cataracts form as a result of aging known as senile cataract. The formation of senile cataract involves a number of physiological factors, one of which is oxidative damage. Hydrogen peroxide (H₂O₂) a non radical member of the active oxygen has been shown to be toxic to the lens (1,2). The active oxygen species have subsequently been shown to induce lipid peroxidation (3,4) as well as oxidation and aggregation of lens protein.

Studies have been conducted on the mechanisms concerned with protecting the lens against the toxic effects of reactive oxygen species. The tissue has been shown to contain enzymes such as superoxide

dismutase, catalase, glutathione peroxidase (5) which form the first line of defense against the oxygen onslaught. These enzymes cannot act together as they are distributed differentially in the cellular components. Investigations on the effectiveness of other defenses against oxidative stress were thus considered important. In the lens, ascorbate and glutathione are such important antioxidants. However the contents of these compounds depend on the dietary intake of vitamin C and the sulphur containing amino acids, also, the enzymatic and the non enzymatic systems lose their efficiency in cataract. The objectives of the present study were to explore the possibilities of peroxidative degradation of lipids that might be occurring concurrent with the "in vitro" development of cataract induced by high levels of H₂O₂ and their possible prevention by the endogenous physiological antioxidants. The extent of peroxidative degradation was assessed by malondialdehyde (MDA) measurement. The specific activity of glutathione peroxidase (G-Px) was also examined in order to assess its role against H₂O₂ in experimental cataractous lenses in the presence and absence of ketoacids as antioxidants.

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MATERIALS AND METHODS

To study the process of cataractogenesis, a total of 100 fresh goat lenses were analyzed. The eye balls of the goat were obtained from the slaughter house and they were brought to the department of biochemistry in an ice bath. Lenses were removed from the eye balls by intracapsular lens extraction method within one hour of the death of the animal. The wet weight of the lenses were recorded. These lenses were incubated in culture media using 'Lens Organ culture technique'(6).

Experimental design

I) Control groups (20 lenses)

The lenses were incubated individually in TC-199 media at room temperature for 72 hours.

II) Experimental cataract group (20 lenses)

Lenses were incubated individually in TC-199 media for 72 hours. The medium was supplemented with 30% H_2O_2 solution at the final concentration of 10mM.

III) Experimental cataract group and ketoacids (20 lenses each).

Lenses were incubated in TC-199 media with 10mM H_2O_2 and different concentration of following ketoacids (separately) for 72 hours.

Pyruvate(10mM), alphaketoglutarate(20mM) and oxaloacetate(20mM).

At the end of the incubation period, the lenses were removed from the culture media, rolled on filter paper to remove adherent water and zonules and weighed separately. Then the whole lens was homogenized in 0.1 M sodium phosphate buffer pH 7.4 and w/v was adjusted to 20 gm%. The homogenate was centrifuged at 10,000 x g at -4 degree Celsius for 30 minutes in a cool centrifuge. The supernatant fluid was used for the measurement of MDA and activity of G-Px.

Estimation of lipid peroxidation (MDA) level (7)

Reagents for the estimation were of AR grade from Qualigens.

1ml of lens homogenate was reacted with 1 ml of 20% w/v trichloroacetic acid and 2 ml of 0.67% w/v of thiobarbituric acid and mixed well. The mixture was heated in boiling water bath for 10 minutes,

cooled and centrifuged. The absorbance of solution was read at 530 nm and the levels of MDA calculated.

The results were expressed as nmol/gm wet weight of lens.

Assay of G-Px activity (8)

Reagents were obtained from Qualigens and Sigma Co.

0.6 ml of lens homogenate was added to 0.6 ml of phosphate buffer saline (PBS) (pH 7.4), 0.6 ml of freshly prepared reduced glutathione solution(0.2%w/v in PBS), 3.0 ml of ethylenediamine tetraacetic acid(EDTA)- sodium azide solution(0.002M EDTA and 0.005M sodium azide in PBS). The reaction was initiated by rapid addition of 1 ml of freshly prepared H_2O_2 solution (0.0018M) to the above mixture at zero time. Since the reaction follows the first order kinetics for 7/2 minutes, it was convenient to stop the reaction at 1 minute and 3 minutes. Thus, exactly at 1 minute and 3 minutes, 2 ml aliquots were transferred into tube containing 5 ml of metaphosphoric acid(0.7% w/v) and 3 gms of sodium chloride to each tube. The tubes were vigorously shaken for 3 min and the mixture was filtered. The filtrate was used within 30 minutes for the estimation of reduced glutathione(GSH)(9). To 2 ml of filtrate, 2.5 ml of tris buffer (1.0 M. pH8.0) and 0.2 ml of 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) reagent (0.04% w/v in 1% citrate solution) were added. The yellow color developed was measured at 412 nm against blank and activity of G-Px calculated.

The results were expressed as U/mg of protein.

RESULTS AND DISCUSSION

The results of the present study demonstrate that there is a significant elevation in contents of MDA in experimental cataractous lenses as compared to control lenses as shown in Table I. The formation of lipid peroxides in cataractous lenses indicate that the oxidation of lipids may be an integral part of the entire process of cataract formation. Experiments were therefore undertaken to examine the feasibility of preventing lipid peroxidation in the lens by use of ketoacids as antioxidants.

Among the antioxidant enzymes, the selenium containing glutathione peroxidase catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione as hydrogen

donor. The activity of this enzyme was significantly declined in experimental cataractous lenses as compared to control (Table I). H_2O_2 can cause cataract, producing damage to both the cell membrane and cytosol regions as it causes a decrease in the levels of antioxidants, like GSH, present in the aqueous humor(10,11). This occurs because of the ability of GSH to detoxify H_2O_2 via G-Px resulting in the formation of oxidized glutathione, GSSG (12). There is also 'mopping up' of hydrogen peroxide by glutathione in order to prevent the oxidation of the sulphydryl groups present on cysteine residues in proteins - an important mechanism given that such oxidation can lead to protein denaturation. Glutathione is a tripeptide (Glu-Cys-Gly) containing a sulphydryl group and is present at high levels in the cell. It serves as a sulphydryl buffer, cycling between a reduced thiol form (GSH) and an oxidised form (GSSG) in which two tripeptides are linked by a disulphide bond. Usually a high ratio (500:1) of GSH:GSSG ensures an adequate buffering capacity but a vast excess of hydrogen peroxide may lead to exhaustion of the system, and leave proteins prone to oxidative aggregation. Therefore it can be concluded that the decreased activity of G-Px observed in cataractous lenses is the consequence of chronic deficiency in reduced glutathione and not due to inhibition by high concentrations of H_2O_2 .

Lenses subjected to oxidative stress, on addition

of pyruvate, alpha ketoglutarate and oxaloacetate showed a significant decrease in MDA values and significant rise in G-Px activity as compared to experimental cataractous lenses (Table I). It is known pyruvate and other ketoacids are rapidly decarboxylated by H_2O_2 producing acetate, carbondioxide and water (13), all non toxic compounds. The protective effect of these ketoacids has been confirmed by other workers (14,15,16). Other advantages of using these ketoacids are

1. Pyruvate forms acetyl CoA used for beta-oxidation, is glucogenic and can be converted by transamination to glycine and glutamate.
2. Alpha ketoglutarate and oxaloacetate are important in Krebs cycle and also are precursors of amino acids like glutamate and aspartate respectively. They are also glucogenic.
3. Tissue is able to use pyruvate for ATP synthesis.

Thus these ketoacids can be very promising antioxidants not only for the treatment of cataracts, but also against other disabilities related to acute and chronic oxidative stress. The therapeutic efficacy of these endogenous antioxidants by way of their inclusion in anticataractous drugs can be a matter of consideration in formulating pharmacologically suitable anticataract agents.

TABLE I

MDA levels and G-Px activity of control and experimental cataractous lenses without and with addition of ketoacids.

Group	MDA (nmol/wet weight in gms) Mean $\pm$ S.D.	GPx activity (U/mg of protein) Mean $\pm$ S.D.
Control	23.65 $\pm$ 4.98	7.55 $\pm$ 1.84
Experimental H ₂ O ₂ cataract group	36.16 $\pm$ 9.17*	4.47 $\pm$ 1.07**
Experimental cataract group & ketoacids		
1) Pyruvate	15.66 $\pm$ 4.29*	7.45 $\pm$ 1.41*
2) alphaketoglutarate	15.80 $\pm$ 1.50*	7.26 $\pm$ 1.50*
3) Oxaloacetate	15.50 $\pm$ 4.36*	7.49 $\pm$ 1.71*

* p < 0.05

** p < 0.001

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