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Original Research Article

Effect of Endogenous Antioxidants on Hydrogen Peroxide Induced Experimental Cataract

Pradnya Padalkar*, PM Bulakh, RR Melinkeri

Department of Biochemistry, Bharati Vidyapeeth Deemed University Medical College, Pune

*Correspondence Email: pradnya.padalkar79@gmail.com

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ABSTRACT

Cataract is an opacification of the lens of eye. Causes of cataract formation are ageing, generation of free radicals, diabetes etc. Antioxidants & antioxidant enzymes protect the eyes by reducing free radical damage.

Scope: Addition of endogenous antioxidants may delay the progression of cataract in hydrogen peroxide induced experimental cataract.

Material and Methods: Total 120 fresh goat lenses were used for the study.

Lenses were incubated by "Lens organ culture technique" in TC-199 culture media with the addition of antibiotics and antifungal agents and they were divided into following groups…

Group 1(control, 30) = TC-199+ lens

Group 2 (Exptl- H_2O_2,) = TC-199+ lens +H_2O_2(30)

Exptl- Endogenous antioxidant= TC-199+ lens+ H_2O_2+Glutathione (30)

= TC-199+ lens+ H_2O_2+Pyruvate (30)

Lens homogenate was subjected to spectrophotometric measurement of total soluble Lens proteins, MDA, Superoxide dismutase, Catalase & Glutathione peroxidase.

Findings: Addition of endogenous antioxidants (Glutathione & Pyruvate) in culture media Showed decreased MDA levels(nmol/gmwt) 9.77±2.78,10.18±3.46($P<0.0001$) And increased activity of antioxidant enzymes (Units/mg protein) 0.75± 0.33, 0.86± 0.29, 0.92±0.49,1.2±1.0, 33.6±9.7,39.5±14.3($P<0.0001$, $P<0.01$).This change is found to be statistically significant.

Conclusion: Study shows that addition of Glutathione & Pyruvate delays the progression of cataract by reducing free radical formation.

INTRODUCTION

Cataract is one of the leading causes of visual disability leading to blindness in the elderly population. It is defined as any opacity in the lens or its capsule whether developmental or acquired is called cataract.[(1)] Causes of cataract formation are ageing, generation of free radicals, diabetes etc.

Cells have their own supply of endogenous antioxidants for repair of oxidative damage, and depletion of these antioxidants could be the cause for oxidative tissue damage. [(2)]

Superoxide radicals are dismutated by superoxide dismutase into hydrogen peroxide. This hydrogen peroxide is metabolized by catalase into water but this reaction requires reduced glutathione to metabolize hydrogen peroxide. Thus catalase and glutathione peroxidase detoxify H_2O_2 with the help of reduced glutathione into oxidized glutathione and water. There is need to maintain reduced form of glutathione. So Oxidized form of glutathione is converted back into reduced form with the help of glutathione reductase and NADPH.

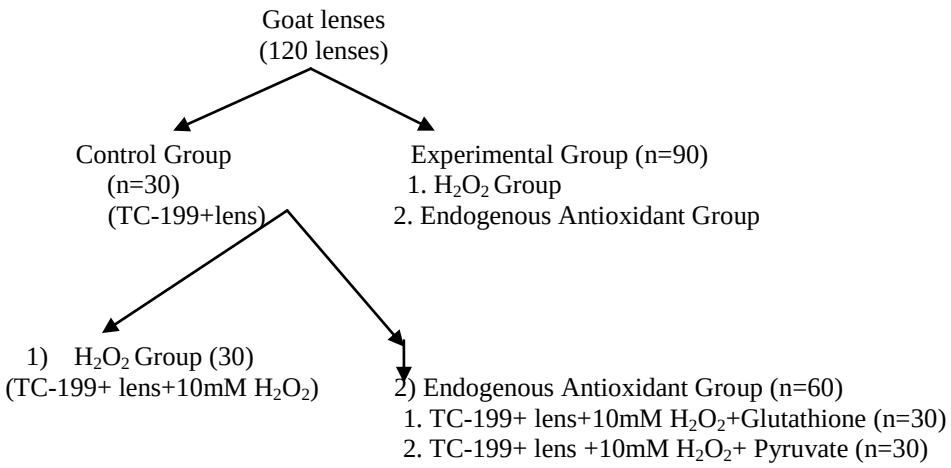
Therefore the following objectives were designed.

1. To study the process of cataractogenesis, by using Hydrogen Peroxide induced experimental cataract by using "Lens organ culture technique".
2. To estimate MDA level as an index of Lipid peroxidation.
3. To estimate the conc. of lens total soluble proteins to studythe biochemical events in cataractogenesis.
4. To study the effect of Glutathione & pyruvate on activity of antioxidant enzymes (SOD, Catalase, GSH-PX).

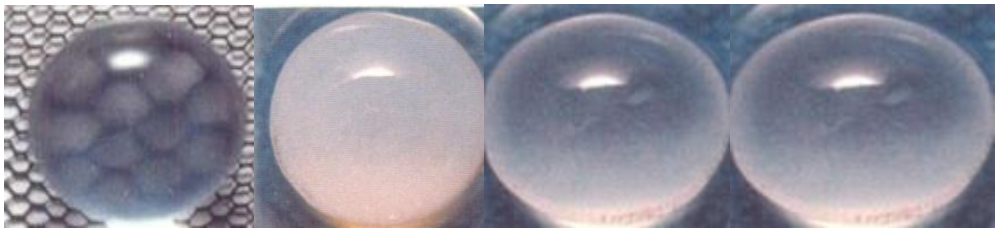
MATERIALS AND METHODS

Hydrogen Peroxide induced cataract" has been chosen as a model for present study. Goat lenses were used for the development of cataract. Eye balls were obtained from slaughter house and transported to the laboratory in ice- box. Lenses were removed from eyeballs by intracapsular lens extraction method & then were weighed separately.

Total 120 goat lenses were used for the study. These lenses were divided under the following groups.



Lenses were incubated in TC-199 culture media for 72 hrs, using "Lens Organ culture technique". [(3)] Penicillin and mycostatin were added in media to prevent bacterial and fungal infection. Lens transparency was measured by observing number of squares seen through the lens & that was also observed for development of cataract, and noted visually & recorded photographically.



Control **H_2O_2** **Glutathione** **Pyruvate**

Homogenate of cultured lens was prepared in 0.1M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 x g for 30 min at – 4^0C. The supernatant was collected and stored at –20^0C until further use. Lens homogenate of each group was subjected to the following estimations,

1. Total soluble proteins (Lowry's method, 1955)
2. MDA levels (Kei Satoh, 1978)
3. Superoxide dismutase (Marklund & Marklund, 1974)
4. Catalase (Aebi, 1974)
5. Glutathione Peroxidase, by Randoxkit (Paglia & valentine,1967)

RESULTS

All data were collected and analysed. P value was calculated by using 't test calculator' software and all results were statistically significant. Total soluble lens proteins shows decrease in H_2O_2 group (193±60.01mg/lens) compared to control group(222.53±48.61 mg/lens), which was increased after the addition of glutathione & Pyruvate (205±59.57mg/lens, 216.35±50.45 mg/lens). MDA levels shows increase in H_2O_2 group (19.70± 4.14nmol/gmwt) than control group (9.99±4.00nmol/gmwt), but addition of glutathione and pyruvate shows decrease in MDA value (10.18± 3.46,9nmol/gmwt.9.77±2.78nmol/gmwt). In case of antioxidant enzymes decrease was observed in H_2O_2group than Control, but addition of glutathione & pyruvate shows increased antioxidant enzymes (0.86±0.29U/mg protein, 1.2±1.0U/mg protein, 39.5±14.3 U/mg protein & 0.75±0.33U/mg protein, 0.92±0.49U/ mg protein, 33.6±9.7U/mg protein).

Table 1: Comparison between control and H_2O_2 group and P value given in table 1.

Group	Wet Weight mg Mean ±S.D	Total soluble lens protein mg/lens Mean ± S.D	MDA nmol/ gm wt Mean ± S.D	SOD Units/mg protein Mean ± S.D	Catalase Units/mg protein Mean ±S.D	GSH-Px Units/mg protein Mean ± S.D
Control group	660 ± 32.8	222.53 ± 48.61	9.99 ± 4.00	0.98 ±0.26	0.95 ±0.68	43.7±15.7
H_2O_2group 10mM	740 ± 41.0 P<0.005	193 ± 60.01 P<0.05	19.70 ± 4.14 P<0.001	0.35 ±0.18 P<0.0001	0.63 ±0.35 P<0.05	21.8±12.6 P=0.003

Table 2: Comparison of H_2O_2 group &endogenous antioxidant group (glutathione and pyruvate).

Group	Wet Weight mg Mean ±S.D	Total soluble protein (mg/lens) Mean± S.D	MDA (nmol/gmwt) Mean± S.D	SOD (Units/mg protein) Mean± S.D	Catalase (Units/mg protein) Mean ± S.D	GSH_PX Units/mg protein Mean± S.D
H_2O_2Gr.	740 ± 41.0	193 ± 60.01	19.70 ± 4.14	0.35 ± 0.18	0.49 ± 0.10	21.8±12.6
H_2O_2+ Pyruvate group (10mM)	710± 35.0 P<0.01	205±59.57 P=0.001	9.77± 2.78 P<0.0001	0.75± 0.33 P<0.0001	0.92 ±0.49 P=0.01	33.6±9.7 P=0.03
H_2O_2+ Glutathione group (100µM)	714± 26.4 P<0.01	216.35±50.45 P=0.0002	10.18± 3.46 P<0.0001	0.86± 0.29 P<0.0001	1.2±1.0 P=0.007	39.5± 14.3 P=0.009

DISCUSSION

Oxidative stress is one of the important factors involved in pathogenesis of cataract. The lens has antioxidant protection system i.e Antioxidant nutrients & antioxidant enzymes to neutarlize the free radicals. [4]

In the present study, MDA levels are significantly raised in experimental cataractous lenses, which could be due to increased free radical generation & depletion of cellular defense mechanism.[5, 6]

Decreased concentration of lens soluble proteins leads to insolublisation of soluble proteins which may be due to oxidative insult. [7]

Present study shows decreased levels of antioxidant enzymes in cataractous lenses. But the addition of endogenous antioxidant (glutathione & pyruvate) shows marked increase in the enzyme levels. The toxic effect of reactive oxygen species may be neutralized by antioxidant defenses.

Important function of Glutathione in the lens is to protect protein thiol group & prevent cross linking of soluble crystallins & thus prevent insolublisation of proteins. [8]

Pyruvate acts as a scavenger of H_2O_2. Pyruvate detoxifies H_2O_2into carbondioxide, water & acetate by nonenzymatic decarboxylation reactions. [9]

CONCLUSION

Finally it is concluded that, addition of glutathione & pyruvate [10] in cultured media may delay the progression of cataractogenesis by reducing free radical formation.

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Illustrations

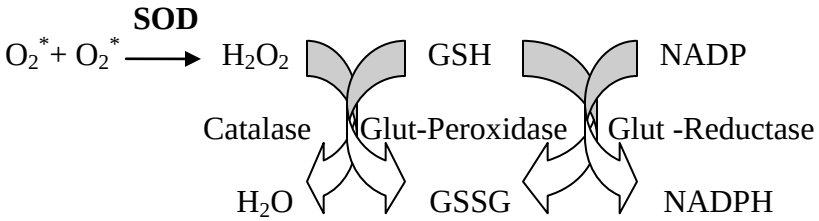

Fig1. Redox cycle involving antioxidant enzymes & their role.

Abbreviations:
1. MDA – Malondialdehyde
2. SOD- Superoxide Dismutase
3. G-Px-Glutathione Peroxidase
4. H_2O_2- Hydrogen peroxide
5. GSH- Reduced glutathione
6. GSSG- Oxidized glutathione

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