Indian Journal of Basic & Applied Medical Research; March 2013: Issue-6, Vol.-2, P. 540-545

"EFFECT OF NSAIDs ON LENS MEMBRANE PERMEABILITY IN EXPERIMENTAL CATARACT"

Umesh More[1], Sarita A.Shinde[1], Arti M. Hajarnavis[2] ,A.N.Sontakke[3],P.M.Bulakh[2]

[1]Department of Biochemistry, Pad.Dr.D.Y.Patil Medical College ,Pune, Maharashtra India.
[2]Bharati Vidyapeet (Deemed University) Medical College, Pune
[3]MIMER Medical College, Talegaon Dabhade, Pune

Abstract:

Introduction: The present study was designed to understand the mechanism of lens protection and biochemical basis of cataract formation.

Methodology: The H_2O_2 induced experimental cataract model was chosen. These lenses were subjected to "Lens organ culture technique". The cultured lenses were categorized as control group and Experimental groups. To study the effect of NSAIDs (Aspirin and Paracetamol) on lens ATPase activity in H_2O_2 induced cataract. The study enrolled total 210 fresh goat lenses were analysed.

Observations : It was observed that concentration of K^+, Mg^{++} and Ca^{++} was significantly decreased and Na^+ concentration was increased in H_2O_2 induced cataractous lenses. In presence of ouabain electrolytes levels were increased. Lenses, on addition of aspirin and paracetamol, showed significantly increased concentration of K^+, Mg^{++} and Ca^{++} whereas the concentration of Na^+ was decreased and the specific activities of ATPase systems showed increase in experimental cataractous lenses. Aspirin and paracetamol in presence of Ouabain, showed decreased concentration of Na^+ and increase in K^+ Ca^{++}concentration. The specific activity of Na^+-K^+ATPase and Mg^{++}-ATPase was increased.

Conclusion: The therapeutic efficacy of these NSAIDs by way of their inclusion in anticataractous drugs is a matter of consideration in formulating pharmacologically suitable anticataract agents.

Key words: Experimental cataract, Nonsteroidal anti-inflammatory drugs (NSAIDs), lens ATPase activity

Introduction: There are numerous causes for development of lens opacities including Physical factors, biochemical factors, Predisposing eye conditions, genetic factors, and Ageing. The biochemical background of cataractogenesis is still unknown. In general, only the initial steps of the involved pathogenic mechanism and the resulting morphological changes due to hydration and / or protein denaturation are known. The membranes play an important role in regulating ions and water movement in the lens. Lens membrane permeability is very important in maintenance of ionic equilibrium in the lens, which depends on the activity of lens Na^+-K^+ATPase and Ca^{++}ATPase system[1]. The plasma membrane of the lens epithelial cells and lens fibre cells are the focal points for damage during cataract formation [2]. The drugs like analgesics and anti-inflammatory delay the loss of transparency of lenses and increase their viability in the normal lens [3,4]. As their mechanism of action is not clearly and definitely established, It would be interesting to study their effect on lens membrane permeability.

Materials and Methods: In the present study, total 210 fresh goat lenses were analysed. These lenses were incubated in a tissue culture medium for 36 hours, using "Lens organ culture technique"[5]. The cultured lenses were categorized as follow: 1.Control groups: (30) Lenses were incubated in TC-199 media 2. Experimental cataract

groups nonsteroidal anti-inflammatory drugs: (60+60) Lenses were incubated in media with 10mM H_2O_2 and different concentrations of Aspirin 300mg and Paracetamol 500mg. At the end of the incubation period of each batch, goat lenses were removed from the culture media, rolled on filter paper to remove adherent water and zonuels and weighed separately. Then the whole lens was homogenized in 0.1M sodium phosphate buffers, pH 7.4 and W/V was adjusted to 10gm%. The homogenate was centrifuged at 10,000 X g for one hour at -4^0C in a refrigerated centrifuge and the supernatant was retained. This supernatant was used for the biochemical analysis. Estimation of electrolytes by Flamephotometry [6] ionized calcium by ISE Method[7], Magnesium[8], Na^+-K^+ ATPase[9] and Ca^{++} ATPase[10] by colorimeter.

Statistical Analysis: Statistical comparison of data was done by paired student's't' test. Values were expressed as mean ± standard deviation (SD).

Observations and Results :

Ionic equilibrium: It was observed that concentration of K^+, Mg^{++} and Ca^{++} was significantly decreased and Na^+ concentration was increased in H_2O_2 induced cataractous lenses. In presence of ouabain, Na^+-K^+ATPase inhibitor, $Na,^+$ K^+ , Mg^{++} and Ca^{++} levels were increased in experimental cataractous lenses (Table No1). Membrane ATPase system: The specific activity of Na^+-K^+ATPase, Mg^{++}-ATPase and Ca^{++}ATPase were found to be significantly decreased in H_2O_2 induced cataractous lenses. In presence of Na^+-K^+ATPase inhibitor, the activity of Na^+-K^+ATPase, Mg^{++}-ATPase and Ca^{++}ATPase activities were significantly decreased in experimental cataractous lenses (Table No2).

Addition of Nonsteroidal anti-inflammatory drugs (NSAIDs) Aspirin**:** Lenses, on addition of aspirin in culture media, showed significantly increased concentration of K^+, Mg^{++} and Ca^{++} and Na^+ concentration was increased in H_2O_2 induced cataractous lenses. the specific activities of all the three ATPase systems showed increase in experimental cataractous lenses.

Aspirin in presence of Na^+-K^+ATPase inhibitor, showed significantly decreased concentration of Na^+ and increase in Ca^{++} concentration. The specific activity of Na^+-K^+ATPase and Mg^{++}-ATPase and Ca^{++}ATPase activity was increased, but no significant changes were noticed.

Paracetamol: Lenses, on addition of paracetamol in the culture media, showed significantly increased concentration of $K,^+$ Mg^{++} and Ca^{++} whereas the concentration of Na^+ was decreased and the specific activities of all the three ATPase system showed increase in experimental cataractous lenses.

paracetamol presence of Na^+-K^+ATPase inhibitor, showed decreased concentration of Na^+ and significant increased in K^+ and Ca^{++}concentration. The specific activities of Na^+-K^+ATPase, Mg^{++}-ATPase were increased and Ca^{++}ATPase activity were decreased.

Discussion :

It is usually very difficult to ascertain the exact locus of the primary disturbance within the multi- complex system of lens metabolism. "Lens Organ Culture," technique were used for evaluating drugs acting on the eye was employed by us with some modification. We have chosen two commonly used drugs as an analgesics and anti-inflammatory Viz. Aspirin and Paracetamol. The effects of these drugs were studied on ionic equilibrium and lens membrane bound enzymes Na^+-K^+ATPase and Ca^{++}ATPase in experimental cataract.

Little controversy exists on the fundamental role played by membranes in regulating ion and water movement in the

different parts of the lens [11,12]. In culture, Na^+ ion extrusion pump also depends upon glucose concentration or metabolism. Disruption of the physicochemical integrity of the membrane or metabolism causes subsequent gain of Na^+ and water, lens swelling and eventually complete loss of lens transparency. (Table No.1.H_2O_2 induced cataract).

The peroxide is formed in aqueous humour during the oxidation of ascorbic acid, which is normally present in high concentration in this fluid. This oxidation reaction has found to be catalysed by light. If H_2O_2 were not detoxified by the lens, oxidative damage to lens membranes and susceptible protein thiol groups would result. It has been suggested that in older human lenses H_2O_2 may be a factor in the development of nuclear cataracts. The epithelium appears to be primarily responsible for the detoxification of H_2O_2. Thus, an impairment of the H_2O_2 detoxification mechanism could considerably result in cataract formation [13]. When experiment was terminated after 36 hours, lenses became opaque and started disrupting. Na^+-K^+ATPase activity was significantly decreased and increased concentration of sodium with concomitant decrease in potassium ion concentration and significant decrease in Na^+-K^+ATPase activity when lenses were cultured with Na^+-K^+ATPase inhibitor (Ouabain) [14], Table No.2.

The loss of calcium homeostasis in the lens plays an important role in cataract formation. Ca^{++}ATPase and membrane lipid permeability are essential to calcium homeostasis. When the lens Ca^{++}ATPase activity was inhibited, an accumulation of calcium in the lens was observed that dysfunction of the Ca – pump system leads to calcium accumulation, thus in turn inducing protein denaturation, which in turn is followed by lens opacification [15,16].

The drugs like analgesics and anti-inflammatory delay the loss of transparency of lenses and increase their viability in the normal lens. As their mechanism of action is not clearly and definitely established, the anti-inflammatory action of the NSAIDs is due mainly to inhibition of cyclooxygenase, and thus prostaglandin synthesis is inhibited. In the present study, addition of aspirin and paracetamol independently in culture media, the lens Na^+-K^+ATPase activity was significantly increased at the end of 36 hours incubation (Table No. 2) in experimental cataract along with addition of ouabain. It has a beneficiary effect on exchange of electrolytes and delay in lens opacification.

The post – synthetic modification such as carbamylation, non – enzymatic glycosylation of lens crystalline were believed to be causative factors in cataractogenesis. The exact mechanism of action of NSAIDs in preventing cataracts is not known, but the possible theory is aspirin, decrease the acetylation ,glycation and carbamylation of lens proteins which may protect the lens proteins [17,18]. The consumption of Paracetamol for at least 4 months is associated with a significant protection against cataract. A mechanism involving prostaglandin is superficially attractive but Paracetamol, which is a feeble inhibitor of prostaglandin synthesis, appears strongly protective against cataract [19]. The topical aspirin possesses significant anticataract activity in galactosemic cataract [20].

Conclusions : The present study conclude that the onset of lens opacities is usually preceded and accompanied by specific changes of its metabolism, a possible drug effect may be studied by monitoring biochemical parameters to pin point the exact metabolic defect. The therapeutic efficacy of these aspirin and paracetamol by way of their inclusion in anticataractous drugs is a matter of consideration in

formulating pharmacologically suitable anticataract agents.

References :

1. David L. Epstein and Kinoshita J.H. The effect of diamide on lens glutathione and lens membrane function. Investigative Opthalmology & Visual Science 1970; 9:629 – 638

2. Cotlier E. ^{86}Rb transport, water and cation concentrations in lenses exposed to polymyxin B sulfate. Exp Eye Res 1973; 15: 711 -722

3. Nishi O., Nishi K., Fujiwara T, Shirasawa E. Effects of diclofenac sodium and indomethacin on proliferation and collagen synthesis of lens epithelial cell in vitro. J Cataract Refract Surg 1995; 21: 461-465

4. Stevens A. The effectiveness of putative anticataract agents in the prevention of protein glycation. J Am Optom Assoc 1995; 66: 744-749

5. Haddad H. M.,Shore B. and Furman Lens organ culture methodology and metabolic evaluation. Amer J Opthalmolo 1967; 63 :1731-1736

6. P.T. Gilbert, R.C.Hawes and A.O. Beckman Beckman Flame Spectrophotometer. Anal Chem 1950;22: 772-780

7. George, G.N.Bowers, Jr C. Brassard and S.F.Sena Measurement of ionized calcium in serum with ion-selective electrodes: a mature technology that can meet the daily service needs. Clinical Chemistry 1986; 32: 1437 – 1447

8. D.W.Neill and R.A.Neely The estimation of magnesium in serum using titan yellow. J Clin Patho 1956; 9:162-163

9. George J.Brewer, John W. Eatox, Clifforde Sodium-potassium stimulated ATPase activity of mammalian hemolysate: Clinical observations and dominance of ATPase deficiency in the potassium polymorphism of sheep. J Lab And Clin Med 1968; 71: 744 – 753

10. Georges Rorive and Kleinzeller The effect of ATP and Ca^{++} on the cell volume in isolated Kidney tubules. Biochim Biophys Acta 1972; 274: 226-239.

11. Kinsey V.E. and Reddy D.V. Studies on the crystalline lens XI. The relative role of the epithelium and capsule in transport. Invest Opthalmology & Visual Science 1965; 4: 104-116

12. Duncan G. Permeability of amphibian lens membrane to water. Exp Eye Res 1970; 9: 188 – 197

13. Pirie A. Glutathione peroxidase in lens and a source of hydrogen peroxide in aqueous humour. Biochem J 1965; 96: 244 – 253

14. Albal M.V. Chandarkar. Bulakh P.M. Effect of inhibition of lens membrane Na^{+}-K^{+}ATPase by ouabai pre-treatment an in vitro study. Indian J Opthalmol 1987; 35: 214 – 216

15. Iimuro A Takehana M. and Iwatas. Influence of calmodulin antagoniets on Ca^{++}

transport in the lens. Ophthalmic Res 1987; 19: 95 - 100

16. Wang Z, Hess J.L.Bunce G.E. Calcium efflux in rat lens: Na/Ca- exchange related to cataract induced by selenite. Current Eye Res 1992; 11: 625 – 632
17. Mihail S. Aspirin in the preventive treatment of cataract. Oftalmologia 1990; 34:43 - 46
18. Rao G.N.,Lardis M.P. and Cotlier E Acetylation of lens cystallins: a possible mechanism by which aspirin could prevent cataract formation. Biochem Biophys Res Commune 1985; 128:1125 - 1132
19. Harding J.J. and Van Heyningen R. Drugs, including alcohol, that act as risk factors for cataract, and possible protection against cataract by aspirin-like analgesics and cyclopenthiazide. British Journal of Opthalmol 1988; 72: 809 - 814
20. Gupta S.K. Joshi S. Tandon R. and Mathur P. Topical aspirin provides protection against galactosemic cataract. Indian J of opthalmol 1997; 45:221 - 225

TABLE 1: CONCENTRATION OF ELECTROLYTES IN H_2O_2 INDUCED CATARACT LENSES ON ADDITION OF NSAIDS WITH AND WITHOUT ADDITION OF Na^+-K^+ATPase INHIBITOR

Group	Na^+ (mmol/ Kg lens Wt Mean ± S.D.	K^+ (mmol/ Kg lens Wt Mean ± S.D.	Mg^{++} (mmol/ Kg lens Wt Mean ± S.D.	Ca^{++} (mmol/ Kg lens Wt Mean ± S.D.
Control	150.90 ±18.81	98.10 ±26.15	1.85 ±0.34	0.33 ±0.08
H_2O_2 induced Cataract	164.00 ±39.78	73.22 ±20.31*	0.82±0.29*	0.22 ±0.02*
H_2O_2 induced Cataract (Without inhibitor)				
1. Aspirin	114.66 ± 12.56***	104.63 ±7.10***	0.98 ±0.21*	0.29 ±0.03***
2. Paracetamol	151.32 ±11.14	101.47 ±8.20***	1.01 ±0.21**	0.34 ±0.03***
H_2O_2 induced Cataract (With inhibitor)	181.36 ±41.04	90.95 ±17.45**	0.86 ±0.29	0.23 ±0.02
1. Aspirin	105.36 ±8.36*	146.35 ±186.96	1.01 ±0.21	0.32 ±0.03**
2. Paracetamol	106.32 ±20.41**	117.52 ±10.26**	1.04 ±0.21	0.37 ±0.03**

** P < 0.001 * P < 0.01

TABLE 2: ACTIVITY OF ATPase IN H_2O_2 INDUCED CATARACT LENSES ON ADDITION OF NSAIDS WITH AND WITHOUT ADDITION OF Na^+-K^+ATPase INHIBITOR

Group	Na^+- K^+ATPase (µmol P/gm Prot/hr) Mean ± S.D.	Mg^{++} ATPase (µmol P/gm Prot/hr) Mean ± S.D.	Ca^{++} ATPase (µmol P/gm Prot/hr) Mean ± S.D.
Control	10.72 ±1.07	10.03 ±0.93	6.44 ±1.82
H_2O_2 induced Cataract	9.54 ±1.01*	8.63 ±0.71*	2.27 ±0.41*
H_2O_2 induced Cataract (Without inhibitor)			
1. Aspirin	13.17 ±2.5**	8.89 ±0.37	3.21 ±0.82
2. Paracetamol	10.48 ±0.76**	8.82 ±0.85	3.16 ±0.82
H_2O_2 induced Cataract (With inhibitor)	8.63 ±1.12	8.07 ±0.69	2.19 ±0.40
1. Aspirin	14.89 ±1.63	9.57 ±1.25*	3.18 ±0.82
2. Paracetamol	11.41 ± 1.14	9.61 ±1.02	3.10 ±0.80

* $P < 0.05$

Date of Submission: 29 December 2012
Date of Provisional acceptance: 08 January 2013
Date of Final acceptance: 16 February 2013
Date of Publication: 05 March 2013
Source of Support: Nil Conflict of Interest: Nil