EFFECT OF NSAIDs ON LENS MEMBRANE PERMEABILITY IN EXPERIMENTAL CATARACT

Umesh More1, Sarita A. Shinde1, Arti M. Hajarnavis2, A.N. Sontakke3, P.M. Bulakh2

1Department of Biochemistry, Pad. Dr. D.Y. Patil Medical College, Pune, Maharashtra India.
2Bharati Vidyapeet (Deemed University) Medical College, Pune
3MIMER 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 H2O2 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 H2O2 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 H2O2 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$_2$O$_2$ 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 zonules 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° C in a refrigerated centrifuge and the supernatant was retained. This supernatant was used for the biochemical analysis.


**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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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\(_2\)O\(_2\) 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\(_2\)O\(_2\) 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\(_2\)O\(_2\) may be a factor in the development of nuclear cataracts. The epithelium appears to be primarily responsible for the detoxification of H\(_2\)O\(_2\). Thus, an impairment of the H\(_2\)O\(_2\) 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 antcataract agents.

References:

15. Iimuro A Takehana M. and Iwatas. Influence of calmodulin antagonists on Ca++

**TABLE 1: CONCENTRATION OF ELECTROLYTES IN H₂O₂ INDUCED CATARACT LENSES ON ADDITION OF NSAIDS WITH AND WITHOUT ADDITION OF Na⁺-K⁺ATPase INHIBITOR**

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺ (mmol/ Kg lens Wt Mean ± S.D.)</th>
<th>K⁺ (mmol/ Kg lens Wt Mean ± S.D.)</th>
<th>Mg⁺⁺ (mmol/ Kg lens Wt Mean ± S.D.)</th>
<th>Ca⁺⁺ (mmol/ Kg lens Wt Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150.90 ±18.81</td>
<td>98.10 ±26.15</td>
<td>1.85 ±0.34</td>
<td>0.33 ±0.08</td>
</tr>
<tr>
<td>H₂O₂ induced Cataract</td>
<td>164.00 ±39.78</td>
<td>73.22 ±20.31*</td>
<td>0.82±0.29*</td>
<td>0.22 ±0.02*</td>
</tr>
<tr>
<td>H₂O₂ induced Cataract (With inhibitor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Aspirin</td>
<td>114.66 ±12.56***</td>
<td>104.63 ±7.10***</td>
<td>0.98 ±0.21*</td>
<td>0.29 ±0.03***</td>
</tr>
<tr>
<td>2. Paracetamol</td>
<td>151.32 ±11.14</td>
<td>101.47 ±8.20***</td>
<td>1.01 ±0.21*</td>
<td>0.34 ±0.03***</td>
</tr>
<tr>
<td>H₂O₂ induced Cataract (With inhibitor)</td>
<td>181.36 ±41.04</td>
<td>90.95 ±17.45*</td>
<td>0.86 ±0.29</td>
<td>0.23 ±0.02</td>
</tr>
<tr>
<td>1. Aspirin</td>
<td>105.36 ±8.36</td>
<td>146.35 ±186.96</td>
<td>1.01 ±0.21</td>
<td>0.32 ±0.03**</td>
</tr>
<tr>
<td>2. Paracetamol</td>
<td>106.32 ±20.41***</td>
<td>117.52 ±10.26*</td>
<td>1.04 ±0.21</td>
<td>0.37 ±0.03**</td>
</tr>
</tbody>
</table>

**P < 0.001  * P < 0.01
### TABLE 2: ACTIVITY OF ATPase IN H₂O₂ INDUCED CATARACT LENSES ON ADDITION OF NSAIDS WITH AND WITHOUT ADDITION OF Na⁺-K⁺ATPase INHIBITOR

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺-K⁺ATPase (µmol P/gm Prot/hr) Mean ± S.D.</th>
<th>Mg⁺⁺ ATPase (µmol P/gm Prot/hr) Mean ± S.D.</th>
<th>Ca⁺⁺ ATPase (µmol P/gm Prot/hr) Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.72 ±1.07</td>
<td>10.03 ±0.93</td>
<td>6.44 ±1.82</td>
</tr>
<tr>
<td>H₂O₂ induced Cataract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Aspirin</td>
<td>9.54 ±1.01*</td>
<td>8.63 ±0.71*</td>
<td>2.27 ±0.41*</td>
</tr>
<tr>
<td>2. Paracetamol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O₂ induced Cataract (Without inhibitor)</td>
<td>13.17 ±2.5**</td>
<td>8.89 ±0.37</td>
<td>3.21 ±0.82</td>
</tr>
<tr>
<td>1. Aspirin</td>
<td>10.48 ±0.76**</td>
<td>8.82 ±0.85</td>
<td>3.16 ±0.82</td>
</tr>
<tr>
<td>2. Paracetamol</td>
<td>8.63 ±1.12</td>
<td>8.07 ±0.69</td>
<td>2.19 ±0.40</td>
</tr>
<tr>
<td>H₂O₂ induced Cataract (With inhibitor)</td>
<td>14.89 ±1.63</td>
<td>9.57 ±1.25</td>
<td>3.18 ±0.82</td>
</tr>
<tr>
<td>1. Aspirin</td>
<td>11.41 ±1.14</td>
<td>9.61 ±1.02</td>
<td>3.10 ±0.80</td>
</tr>
</tbody>
</table>

* P < 0.05

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