IN VIVO AND OCULAR SAFETY STUDY OF DORSOLAMIDE HCL OCULAR INSERT

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Summary

Dorsolamide HCl is a carbonic anhydrase inhibitor widely used in the treatment of glaucoma available in the form of conventional eye drop form. This conventional dosage form is facing certain drawbacks like poor bioavailability, tear turnover, lacrymal drainage and conjunctival absorption. Optimized formulation was evaluated for ocular safety study, In vivo release study, In vivo intra ocular pressure lowering activity. The results revealed that the optimized ocular insert was safe for ocular administration and showed better in vivo intra ocular pressure lowering activity compare to eye drop and control.

Key Words: Dorsolamide HCl, Glaucoma, Ocular

Introduction

Glaucoma is the second cause of vision loss in the world. They are now an estimated 12 million people affected by glaucoma in India [1] and 60.5 million in the world and by 2020 this is expected to be 16 million in India and 79.6 million in the world [2]. Improved methods of screening and drug delivery systems are the urgent need to address this issue. Dorsolamide HCl is a topically active carbonic anhydrase inhibitor developed to circumvent the side effects of acetazolamide [3]. Its ocular formulation has been marketed in USA since 1995 [3]. Dorsolamide HCl available in conventional eye drop and widely used in treatment of glaucoma. But this conventional dosage form is suffering from inherent drawbacks like patient has to take several times [4], allergic reaction [5], limited bio availability [6], tear dilution, solution drainage [7], tear turnover and conjunctival absorption [8]. Here an attempt was made to circumvent the drawbacks associated with conventional dosage form.

Materials and Methods

Dorsolamide HCl was received as gift sample from INTAS pharmaceutical, Ahmedabad, Dialysis membrane was procured from Himedia laboratories, schioetz tonometer. Other chemicals and solvents used in this study were of analytical grade.
OCULAR SAFETY STUDY
To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). The sterile optimized formulation will placed in one eye of each rabbit by gently pulling the lower eyelid. The eyelids were then being gently held together for one second and animal will released. The other eye remaining untreated was served as the control. The eye of each rabbits will examined 24, 48 and 72 hrs. After treatment for irritation, inflammation by necked eye or by means of pen torch. At the time of examination period rabbits will scored for ocular reaction as per standards mentioned in Table 1, 2 and 3.

IN VIVO RELEASE STUDY
To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). Total seven Albino rabbit of either sex was used in the experiment weighing 2.5 to 3.5 Kg. Inserts were sterilized by using UV radiation for one to two minutes before the study. Albino rabbits of either sex were selected for experiment. The animals were housed individual cages and customized to laboratory condition for a day and received free access to food and water. On the day of experiment the sterilized ocuserts were placed into the lower cul-de-sac of rabbits. The inserts were inserted into one eye of seven rabbits. After 1, 2, 4, 6, 10, 22 and 24 hrs, the inserts were carefully removed and analyzed for remaining drug content by UV Spectrophotometer. The amount of drug release was calculated by subtracting the remaining amount of drug from initial amount of drug. Observations for any fall out of insert were also recorded throughout the experiment[10].

IN VIVO INTRA OCULAR PRESSURE LOWERING ACTIVITY
To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). In vivo intra ocular pressure lowering activity of selected Ocusert preparation of Dorsolamide HCl was studied in normotensive albino rabbits of either sex weighing 2 to 3.5 Kg. The animals were housed under well controlled conditions of temperature (22± 2 °C), humidity (55±5%) and 12/12 – h, light-dark cycle, was given access to food and water. To induce acute glaucoma, 5% dextrose solution (15 ml/kg) was intravenously infused through marginal ear vein. The basal intraocular pressure was measured by schioetz tonometer. The drug formulations were placed at cul-de-sac to rabbits. Total 12 rabbits were divided in to three groups each contains 4 rabbits. In first group marketed preparation of Dorsolamide HCl eye drop equivalent to 0.45 mg was administered. In second group placebo film was inserted which acted as control. In third group medicated film of Dorsolamide HCl was inseted in to lower cul de sac of rabbits. In all cases the preparation was administered after 15 minutes of dextrose injection. The intraocular pressure (IOP) changes were recorded every 30 min till the pressure difference between the control eye and treated eye is zero. Intraocular pressure (IOP) was measured by tonometry method with the help of schioetz tonometer and mean was taken at three times fixed interval. All IOP measurements were carried out by the same operator, using same schioetz tonometer. Each rabbit was given washout period of three days after every treatment. The ocular hypotensive activity was expressed as the average difference in IOP between the treated and control eye of the same rabbit[11,12].

Results and Discussion
**Table 1: Twenty fourth Hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocsert**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Observations</th>
<th>Rabbit No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CORNEA</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>A Scattered or diffused area-details of iris clearly visible</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B Easily discemible translucent areas, details of iris slightly obscured</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C Opalescent areas, no details of iris visible, size of pupil barely discernible</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D Opaque, iris invisible</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E Area of cornea involved</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>IRIS</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B No reaction to light hemorrhage; gross destruction (if any or all of these)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C Score equals – A×B×5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>CONJUNCTIVA</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A Redness (refers to palpebral conjunctiva only)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B Any swelling above normal (include nictitating membrane)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C Any amount different from normal (doesn’t include small amount observed in inner cul-de-sac)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>D Discharge</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>E Discharge with moistening of the lids and hairs just adjacent to the lids</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F Discharge with moistening of the lids and considerable area around the eye</td>
<td></td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>G Score equals – (A+B+C)×2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2: Thirty sixth hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocusert

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Observations</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CORNEA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Opacity - Degree of density (area which is more dense taken for reading)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Scattered or diffused area-details of iris clearly visible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Easily discernible translucent areas, details of iris slightly obscured</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Opalescent areas, no details of iris visible, size of pupil barely discernible</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Opaque, iris invisible</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Area of cornea involved</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>One quarter (or less) but not zero</td>
<td></td>
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<tr>
<td></td>
<td>Greater than one quarter-less than one-half</td>
<td></td>
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<td></td>
<td>Greater than one half less than three quarters</td>
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<td></td>
<td>Greater than three quarters up to whole area</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Score equals – A×B×5</td>
<td>Total possible maximum = 80</td>
<td>Total Score</td>
<td>0</td>
</tr>
<tr>
<td><strong>IRIS</strong></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Values</td>
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<tr>
<td></td>
<td>Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)</td>
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<tr>
<td></td>
<td>Score equals – A×5</td>
<td>Total possible maximum = 10</td>
<td>Total Score</td>
<td>0</td>
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<tr>
<td><strong>CONJUNCTIVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Redness (refers to palpebral conjunctiva only)</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Vessels definitely injected above normal</td>
<td></td>
<td></td>
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<td></td>
<td>More diffuse, deeper crimson red, individual vessel not easily discernible</td>
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<tr>
<td></td>
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<td></td>
<td>Chemosis</td>
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<td></td>
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<tr>
<td></td>
<td>Score equals – (A+B+C)×2</td>
<td>Total possible maximum = 20</td>
<td>Total Score</td>
<td>0</td>
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</table>
Table 3: Seventy two hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocuser
t

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Observations</th>
<th>1</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CORNEA</td>
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<td>A - Opacity - Degree of density (area which is more dense taken for reading)</td>
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<td></td>
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<td>2</td>
<td>IRIS</td>
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<td></td>
<td>A - Values</td>
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<tr>
<td></td>
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<td></td>
<td>Total possible maximum = 10</td>
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<td></td>
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<tr>
<td>3</td>
<td>CONJUNCTIVA</td>
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<td></td>
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<tr>
<td></td>
<td>A - Redness (refers to palpebral conjunctiva only)</td>
<td>1</td>
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<td>Vessels definitely injected above normal</td>
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<td></td>
<td>B - Chemosis</td>
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<td>Any swelling above normal (include nictitating membrane)</td>
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<td>Swelling with lids about half closed</td>
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<tr>
<td></td>
<td>Swelling with lids about half closed to completely closed</td>
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<td></td>
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<tr>
<td></td>
<td>C - Discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any amount different from normal (doesn’t include small amount observed in inner cul-de-sac)</td>
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<td></td>
<td>Discharge with moistening of the lids and hairs just adjacent to the lids</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Score equals – (A+B+C)x2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total possible maximum = 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Score</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
The ocular safety study observations are summarized in table – 1, 2 and 3. The ocular safety score of the optimized formulation was found to be 2 at the end of 72 hours and therefore, considered as practically not irritating. Thus, it can be concluded that they were safe for ocular administration.

IN VIVO RELEASE STUDY OF DORSOLAMIDE HCL OCULAR INSERT

Table 4: In Vivo Drug Release Data of Optimized Dorsolamide HCl ocular insert.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% drug released</th>
<th>In vitro % drug released</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.01±0.35</td>
<td>3.26±0.35</td>
</tr>
<tr>
<td>2</td>
<td>8.1±0.52</td>
<td>6.7±0.51</td>
</tr>
<tr>
<td>4</td>
<td>15.8±0.48</td>
<td>13.01±0.79</td>
</tr>
<tr>
<td>6</td>
<td>23.56±0.89</td>
<td>20.36±0.87</td>
</tr>
<tr>
<td>10</td>
<td>39.25±1.15</td>
<td>35.26±1.01</td>
</tr>
<tr>
<td>22</td>
<td>90.78±0.45</td>
<td>86.47±0.85</td>
</tr>
<tr>
<td>24</td>
<td>100.01±0.69</td>
<td>95.56±0.76</td>
</tr>
</tbody>
</table>

Labeled Claim=0.450 mg
The results of in vivo release study of the optimized formulation is shown in Table 4 and figure 2. The ocusert showed 95.56% of drug release after 24 hours which was comparable to in vitro drug release (table 4). Thus there was good in vitro – in vivo correlation for the optimized formulation(figure - 2) indicating the effectiveness of the formulation to be used in vivo.

### INTRAOCULAR PRESSURE LOWERING ACTIVITY

**Table 5: Intraocular pressure lowering activity of Dorsolamide HCl ocular insert.**

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Control* (mmhg)</th>
<th>Marketed* (mmhg)</th>
<th>Ocusert* (mmhg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.1±0.10</td>
<td>33.0±0.20</td>
<td>33.1±0.14</td>
</tr>
<tr>
<td>30</td>
<td>33.2±0.25</td>
<td>30.4±0.10</td>
<td>30.4±0.19</td>
</tr>
<tr>
<td>60</td>
<td>32.8±0.16</td>
<td>23.8±0.25</td>
<td>26.1±0.25</td>
</tr>
<tr>
<td>90</td>
<td>32.7±0.36</td>
<td>17.0±0.29</td>
<td>22.3±0.37</td>
</tr>
<tr>
<td>120</td>
<td>33.1±0.41</td>
<td>15.4±0.25</td>
<td>20.1±0.45</td>
</tr>
<tr>
<td>150</td>
<td>33.1±0.20</td>
<td>18.5±0.16</td>
<td>16.2±0.35</td>
</tr>
<tr>
<td>180</td>
<td>32.8±0.13</td>
<td>23.2±0.42</td>
<td>15.5±0.17</td>
</tr>
<tr>
<td>210</td>
<td>32.7±0.19</td>
<td>23.8±0.25</td>
<td>15.5±0.18</td>
</tr>
<tr>
<td>240</td>
<td>27.1±0.29</td>
<td>17.3±0.25</td>
<td>14.1±0.42</td>
</tr>
<tr>
<td>270</td>
<td>26.9±0.25</td>
<td>17.0±0.38</td>
<td>14.1±0.32</td>
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<tr>
<td>300</td>
<td>24.8±0.23</td>
<td>15.6±0.10</td>
<td>12.9±0.14</td>
</tr>
<tr>
<td>330</td>
<td>24.5±0.15</td>
<td>15.6±0.19</td>
<td>12.9±0.16</td>
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</table>

Mean ± SD Mean ± SD (*n=3)
It was also observed that at the end of 240 min the effect of all the formulation was found to be nil as it was evident from the figure 3.

Conclusion

It was concluded that prepared Dorsolamide HCl ocular insert is safe for ocular administration as it provide good in vitro in vivo correlation and better in vivo intra ocular pressure lowering activity. Still it is required to evaluate its efficacy by other clinical study.

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References