Effect of preservative, antioxidant and viscolizing agents on \textit{in vitro} transcorneal permeation of ketorolac tromethamine

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The influence of formulation additives, e.g. preservative, antioxidant and viscolizing agents on \textit{in vitro} transcorneal permeation of ketorolac tromethamine from 0.5\% (w/v) aqueous drop was studied using goat cornea. Permeation characteristics of drug, from selected formulations, through excised rabbit cornea were also evaluated. Aqueous solution of ketorolac tromethamine (0.5\% w/v), pH 6.5 or 7.0 having ionic strength 0.2, was prepared. To this solution preservatives either alone or in combination with other additives were added to have drops of various composition. Permeation studies with goat cornea showed maximum permeation of ketorolac tromethamine from formulation containing benzalkonium chloride and disodium edetate. Increase in viscosity of drop resulted in decreased permeation of drug. Formulation containing benzalkonium chloride and disodium edetate also increased permeation of drug through rabbit cornea. Cumulative permeation of drug through rabbit cornea was found to be 2.3-2.4 fold higher than that observed with goat cornea.

Topical therapy with corticosteroids may be an indispensable modality in the treatment of ocular inflammatory disorders but their use is often associated with increase in intraocular pressure, cataract formation and the risk of infection\textsuperscript{1}. Currently many non-steroidal anti-inflammatory drugs (NSAIDs) are being tried as ocular anti-inflammatory agents\textsuperscript{2\textendash}4 so as to diminish the well documented ocular side effects caused by corticosteroids. When applied topically NSAIDs often cause ocular irritation.

Ketorolac tromethamine (KT), an aryl acetic acid derivative NSAID, is non-irritating to the eye at 0.5\% w/v concentration\textsuperscript{5}. Ocular drop of KT is an effective and safe anti-inflammatory agent for topical use following cataract surgery and intraocular lens implantation\textsuperscript{6}. KT is also known to be a viable alternative to corticosteroids in treating ocular inflammation in presence of pathogens\textsuperscript{7\textendash}8. Ophthalmic solution of KT (0.5\%) has been shown to be effective in treatment of chronic aphakic and pseudophakic macular edema\textsuperscript{9}. Transcorneal permeation of the drug from KT (0.5\% w/v) aqueous solution has been reported\textsuperscript{10}. Preservatives are included as a major additive in multiple-dose ophthalmic solutions for the primary purpose of maintaining sterility of the formulation during use. Ophthalmic solutions may also contain antioxidants or chelating agents for better stability of drug. Bioavailability of drug from topically applied ophthalmic solution is very low due to precorneal loss processes that exist at the site of drug delivery. This can be overcome by increasing the viscosity of the instilled solution by incorporation of water-soluble polymers\textsuperscript{11}. Keeping the above in view, the effects of formulation additives e.g. preservative, antioxidant/chelating agent and viscolizing agents on \textit{in vitro} transcorneal permeation of KT from 0.5\% w/v aqueous drop have been studied using excised goat cornea. Permeation characteristics of KT from selected formulations, through excised rabbit cornea, have also been evaluated.

Materials and Methods

Ketorolac tromethamine was obtained from Ranbaxy Laboratories (India). Preservatives and antioxidants were received as gifts from Max India Limited and Panacea Pharmaceuticals Limited (India). Viscolizing agents were gifted by Panacea Biotech Limited (India). All other chemicals used were of analytical reagent grade. Fresh whole eye balls of goat (6-7 months old) were obtained from butcher's shop (Ambedkar Nagar, Delhi), within an hour of slaughtering of the animal. Albino rabbits weighing 2-2.5 kg were obtained from Lucky Zoological House, New Delhi (India). The rabbits were sacrificed by intravenous injection of phenobarbital (Rhone-Poulenc (India) Limited) and eyeballs were removed immediately. The method of dissection of cornea and the apparatus

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used in permeation studies were same as described earlier

**Permeation experiment**—Freshly excised goat cornea was mounted by sandwiching the surrounding scleral tissue between clamped donor and receptor compartments of an all glass diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.47 cm². The receptor compartment was filled with 10 ml freshly prepared balanced base buffer solution (pH 7.4) and all air bubbles were expelled from the compartment. An aliquot (1 ml) of test solution was placed on the cornea and opening of donor cell was sealed with a glass cover slip, while the receptor fluid was kept at 37°C with constant stirring. Permeation was continued for 120 min and samples were withdrawn from receptor, diluted with 0.1N HCl and analysed for ketorolac content by measuring absorbance at 313 nm in a spectrophotometer (Cecil UV-Visible Spectrophotometer)

Complete permeation characteristics of KT from formulations having higher permeation were evaluated by withdrawing 2 ml samples from receptor at 15, 30, 60, 90 and 120 min, replacing withdrawn samples with fresh balanced base buffer solution. Samples were analysed for ketorolac content as described above. At the end of the experiment each cornea (freed from adhering sclera) was weighed, soaked in 1 ml methanol overnight, dried at 90°C and reweighed. From the difference of weights corneal hydration (%) was calculated. Permeation characteristics of KT from selected formulations through excised rabbit cornea were also evaluated. Statistical calculations were done by Student’s t test

**Preparation of test solution**—Aqueous solution of KT, 0.5% (w/v) was formulated in glass distilled water and the resulting solution was adjusted to either pH 6.5 or 7 using 0.1 N NaOH and 0.1N HCl. The ionic strength (μ) of the solution was maintained at 0.2 with NaCl. The preservatives either alone or in combination with different antioxidants were added to the formulation at conventionally prescribed concentrations (w/v) given below. Formulations containing thiomersal or parabens had pH of 7 while the rest had pH of 6.5.

(a) Quaternary ammonium compound : Benzalkonium chloride (BAC, 0.01%)
(b) Organic mercurials : Phenyl mercuric nitrate (PMN, 0.002%), phenyl mercuric acetate (PMA, 0.002%), thiomersal (THM, 0.005%)
(c) Substituted alcohol : Chlorbutol (CB, 0.5%)
(d) Parahydroxy benzoates or parabens : Methyl paraben sodium (MP, 0.1%), propyl paraben sodium (PP, 0.04%)
(e) Antioxidant/reducing agent/chelating agents : Sodium sulphite (SS, 0.1%), sodium metabisulphite (SMS, 0.1%), ascorbic acid (AA, 0.01%), disodium edetate (EDTA, 0.01%), citric acid (CA, 0.02%)

KT drops (0.5%) containing BAC (0.01%) and EDTA (0.01%) were also formulated with viscosizing agents like polyvinyl alcohol (PVA, 1.4%), polyvinylpyrrolidone (PVP, 1%), hydroxypropyl methylcellulose (HPMC, 1%) and methylcellulose (MC, 0.5%). Viscosity of the drops was determined using Ostwald viscometer.

**Results and Discussion**

Initial permeation studies with 0.5%(w/v) KT drops containing preservatives through goat cornea, showed reduced permeation of drug in presence of THM (alone or in combination with CA or AA or SS) or parabens compared to that observed with formulation containing no additive. Formulations containing either BAC, EDTA, CB, PMA, PMN, or SMS indicated enhanced permeation of KT. Complete permeation characteristics of KT from these formulations are shown in Table 1. Permeation of KT from formulation containing no additive had a lag phase of 30 min whereas formulation with either of the preservative and/or antioxidants had a lag phase of 15 min. Among all the preservatives, BAC provided higher permeation of KT followed by CB. Combination of BAC and EDTA however provided maximum permeation of the drug. BAC has been reported to increase in vitro permeation of ketorolac through rabbit cornea and the mechanism suggested for the same are (1) formation of a more lipid soluble ion pair and (2) disruption of corneal epithelium. Results of the present study also indicate higher permeation of KT through goat cornea from drop containing BAC. Presence of BAC and EDTA in formulation increased the permeation to the maximum. EDTA, a known calcium-chelating agent, has been shown to act on cell junctions by interfering with calcium ions and altering intercellular integrity. EDTA also disrupts plasma membrane and consequently increases intercellular permeability. Thus it seems likely that BAC and EDTA combination would increase corneal permeation of KT. The hydration
level of normal mammalian cornea is between 75-80% (ref. 17). Earlier experiments on freshly excised goat cornea (untreated) showed hydration level (%) of 79.2±4.01 (ref. 18). Corneal damage usually increases corneal hydration to 83-92% i.e. 3-7% units or more than the normal value16. Formulation containing BAC and EDTA showed corneal hydration below 80%. Thus 0.5% (w/v) KT drop containing BAC and EDTA does not appear to cause any undue damage to cornea.

Effects of different viscolizing agents on permeation characteristics of KT from 0.5% (w/v) KT drops containing BAC and EDTA through goat cornea were studied next. Results (Table 2) indicate that permeation of KT from drops containing either of PVP, PVA, HPMC or MC was less than that observed with the formulation containing no viscolizing agent. As the viscosity of drop increased the permeation of KT decreased. KT drop containing PVP provided least viscosity and higher corneal permeation of drug while drop formulated with MC had maximum viscosity and least permeation. Increase in viscosity of formulation would decrease diffusion coefficient of the drug and the same could result in reduced permeation.

In vitro permeation studies through goat cornea indicated maximum permeation of KT from formulation containing BAC and EDTA. But, rabbit cornea is considered more close to human cornea and therefore permeation studies conducted with rabbit cornea are better accepted. So permeation characteristics of drug, from KT 0.5% (w/v) drops formulated with or without BAC (0.01%) and EDTA (0.01%) were evaluated using excised rabbit cornea. Paired corneas were used for the experiments, i.e., from a single rabbit, one cornea was treated with the formulation of interest (KT drop containing BAC and EDTA) while the other

Table 1 — In vitro permeation characteristics of KT from 0.5% (w/v) KT drops containing selected preservatives and/or antioxidants through goat cornea

<table>
<thead>
<tr>
<th>Formulation containing</th>
<th>Cumulative permeation (mg)</th>
<th>Cumulative permeation (%)</th>
<th>Corneal hydration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>No additive3</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>BAC</td>
<td>0.014 ± 0.00</td>
<td>0.026 ± 0.00</td>
<td>0.038 ± 0.00</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.013 ± 0.00</td>
<td>0.025 ± 0.00</td>
<td>0.037 ± 0.00</td>
</tr>
<tr>
<td>BAC+EDTA</td>
<td>0.012 ± 0.00</td>
<td>0.024 ± 0.00</td>
<td>0.036 ± 0.00</td>
</tr>
<tr>
<td>PMA</td>
<td>0.014 ± 0.00</td>
<td>0.026 ± 0.00</td>
<td>0.038 ± 0.00</td>
</tr>
<tr>
<td>SMS</td>
<td>0.013 ± 0.00</td>
<td>0.025 ± 0.00</td>
<td>0.037 ± 0.00</td>
</tr>
<tr>
<td>SMS+EDTA</td>
<td>0.012 ± 0.00</td>
<td>0.024 ± 0.00</td>
<td>0.036 ± 0.00</td>
</tr>
<tr>
<td>CB</td>
<td>0.015 ± 0.00</td>
<td>0.027 ± 0.00</td>
<td>0.039 ± 0.00</td>
</tr>
</tbody>
</table>

1Paired corneas were used i.e., from a single goat, one cornea was treated with KT drop containing BAC and EDTA and the other cornea was treated with drop containing no additive.

Table 2 — In vitro permeation characteristics of KT from 0.5% (w/v) KT drops containing combination of BAC and EDTA with different viscolizing agents through goat cornea

<table>
<thead>
<tr>
<th>Viscolizing agent</th>
<th>Viscosity (cps)</th>
<th>Cumulative permeation (mg)</th>
<th>Cumulative permeation (%)</th>
<th>Corneal hydration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>None</td>
<td>0.877</td>
<td>0.013 ± 0.00</td>
<td>0.025 ± 0.00</td>
<td>0.037 ± 0.00</td>
</tr>
<tr>
<td>PVA</td>
<td>2.440</td>
<td>0.0216 ± 0.00</td>
<td>0.0432 ± 0.00</td>
<td>0.0648 ± 0.00</td>
</tr>
<tr>
<td>PVP</td>
<td>1.013</td>
<td>0.0233 ± 0.00</td>
<td>0.0456 ± 0.00</td>
<td>0.0679 ± 0.00</td>
</tr>
<tr>
<td>HPMC</td>
<td>3.550</td>
<td>0.0158 ± 0.00</td>
<td>0.0316 ± 0.00</td>
<td>0.0474 ± 0.00</td>
</tr>
<tr>
<td>MC</td>
<td>12.20</td>
<td>0.0163 ± 0.00</td>
<td>0.0326 ± 0.00</td>
<td>0.0490 ± 0.00</td>
</tr>
</tbody>
</table>

*Significantly different (P<0.05) from the control (no viscolizing agent).
cornea was treated with control formulation (KT drop without any additive). Results are shown in Fig. 1 where the permeation profiles of KT in the presence or absence of BAC and EDTA through paired goat corneas are also included for comparison. Cumulative percentage permeation of KT from 0.5% w/v drop (control) through rabbit cornea was 4.7%. Addition of BAC and EDTA in the drop resulted in 2.7-fold increase in permeation (12.54%). Corneal hydration (rabbit) observed with drop containing BAC and EDTA was 80.27% against 76.72% with control formulation, indicating no corneal damage. Thus the formulation with BAC and EDTA increased the permeation of KT through both goat and rabbit corneas and the increase was statistically significant from 15 min onwards ($P < 0.05$). Cumulative permeation of KT through rabbit cornea was 2.3-2.4 fold higher than that observed with goat cornea indicating higher permeability through rabbit cornea. Madhu et al. have evaluated the in vitro ocular bioavailability of KT from 0.5% KT drops (pH 7.4) with or without BAC (0.01%) and EDTA (0.1%), through rabbit cornea. They have observed increase in ocular bioavailability of KT from drop containing BAC and EDTA but the increase was not statistically significant. The usual concentration of BAC used in topical eye drop is 0.01%. Some strains of *Pseudomonas aeruginosa* have been found that are resistant to BAC and, in fact, can be grown in concentrated solution of this agent. The acquired resistance could be eliminated by the presence of EDTA in the solution. The use of EDTA where it is compatible is recommended in concentrations of 0.01 to 0.1% (ref. 21). Thus to potentiate antibacterial effect of BAC (0.01%), EDTA was included in the formulation used in the present study. The concentration of EDTA was kept at minimum level (i.e., 0.01%) to have minimum damage to the cornea. Earlier studies on in vitro transcorneal permeation of KT have indicated higher permeation of drug at pH 6.5 compared to that observed at pH 7.5. Considering the same, KT 0.5% (w/v) solution, pH 6.5, containing BAC (0.01%) and EDTA (0.01%) was prepared and the formulation significantly increased the permeation of drug through both excised goat and rabbit corneas. The corneal diffusion apparatus used by Madhu et al. was similar to that used in the present studies. But the donor chamber after dosing of drug solution (150 μl) and 100 μl glutathione bicarbonate ringer buffer (GBR) was infused with GBR buffer at a flow rate of 28 μl/min throughout the experiment (i.e., 240 min). Thus the effective concentration of BAC and EDTA on the cornea would decrease with passage of time and the same could possibly account for insignificant increase in permeation of KT. Whereas in the present study 1 ml drug solution was kept on the cornea throughout the experiment (i.e., 120 min) and therefore the effect of BAC and EDTA would be more pronounced resulting in significant increase in permeation of drug.

Thus on the basis of results available it can be concluded that KT 0.5% (w/v) aqueous drop containing BAC (0.01% w/v) and EDTA (0.01% w/v) provides maximum in vitro permeation of KT through goat cornea. Increase in viscosity of drop by addition of viscolizing agent however reduces permeation of drug. KT 0.5% (w/v) drop containing BAC and EDTA also increases permeation of drug through rabbit cornea. Permeability of KT through rabbit cornea is 2.3-2.4 fold higher than that observed with goat cornea.

**Acknowledgement**

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**References**

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