Development, characterization and investigation of anti-inflammatory potential of valdecoxib topical gels

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This study presents formulation of topically effective controlled release valdecoxib gels and evaluates potential of gels as carriers for delivery of valdecoxib. In vitro drug release pattern for all formulations were found to be zero order diffusion controlled release. All formulations were compatible with skin and stable as per ICH guidelines. Optimized valdecoxib gel significantly reduced paw oedema up to 79.51% after 6 h, whereas it was found nonsignificant to inhibit 5-lipoxygenase even though with more doses. Valdecoxib gel with 0.5 g Carbopol is found optimized formulation to treat local inflammation.

Keywords: Cycloxygenase-2, 5-lipoxygenase, In vitro drug release, Skin, Valdecoxib gel

Introduction

In topical drug delivery, drug loaded formulation is applied on skin directly to treat cutaneous manifestations of a general disease1,2. Topical route avoids first pass effects, gastro-intestinal irritation, metabolic degradation associated with oral administration of drug3, less greasy nature and ease in its removal from skin4. To bypass these disadvantages, gel formulations have been proposed as topical drug delivery system. When dispersed in an appropriate solvent, gelling agents merge or entangle to form a three-dimensional colloidal network structure, which limits fluid flow by entrapment and immobilization of solvent molecules5. Non-steroidal anti inflammatory drugs (NSAIDs) show actions by interfering cyclo-oxygenase and lipoxygenase pathways, which are responsible for inflammatory disorders. Arachidonic acid (AA) is converted to prostaglandins (PGs) by cyclooxygenase (COX-1 and COX-2)6,7. COX-1 is constitutive, and present in most of tissues and controls normal body functions8. COX-2 is involved in producing PGs for an inflammatory response9, whereas in lipoxygenase pathway, arachidonic acid is transformed into leukotrienes (LTs) by 5-lipoxygenase, which results in chronic inflammatory conditions10. Valdecoxib, 4-(5-methyl-3-phenyl- isoxazolyl) benzene sulfonamide, is widely used NSAID, which exhibits anti inflammatory, analgesic and antipyretic activities11,12. Oral side effects of valdecoxib pose increased risk of cardiovascular complications and life threatening gastrointestinal bleeding13.

This study presents valdecoxib (Fig. 1) using topical route by developing various polymeric gels and also studies its extent to inhibit COX as well as LOX pathways.

Experimental Section

Valdecoxib was provided by Hetero International, Mumbai. Methylcellulose (MC), Carbopol 934, Hydroxypropylmethyl cellulose (HPMC), mentha oil, triethanolamine (TEM) and all other chemicals were purchased from SD Fine Chem Pvt Ltd, Mumbai. All chemicals were of analytical grade and used without further purification. For UV analysis and in vitro release study, double distilled water was used.

Preparation of Gels

Polymeric dispersion techniques were used to prepare various gel formulations14. Required quantity of drug was dissolved in propylene glycol and then hydroalcoholic vehicle (2:1) was added. In resulting solution, non toxic gelling agents [MC, Carbopol 934 and HPMC] in varying concentrations (0.5, 1 and 2 g) were
dispersed individually to form different homogeneous gels. Penetration enhancer and other excipients (methyl paraben and propyl paraben) were also added with continuous stirring. pH of all formulations was adjusted to skin pH by addition of TEM. Entrapped air bubbles, if any, were removed by keeping gels in vacuum oven (Model: Acm-22068-I, Acmas, India) for 2h.

Evaluation of Gels
Formulated gels were evaluated visually for appearance, presence of any clog and sudden viscosity changes. Gels were taken on glass slide to form a smear, which was observed by microscope for the presence of any clog. Formulations were applied on skin and the feel was experienced psychorheologically. Drug content was estimated spectrophotometrically as per reported method, where formulation (0.5 g) was diluted with 1% w/v sodium lauryl sulphate (SLS) solution, and then shaken to dissolve properly and filtered through whatman filter paper (No.1). Filtrate was estimated spectrophotometrically at $\lambda_{max}$, 239 nm. Viscosity was measured at room temperature (RT) using a programmable cone and plate rheometer (Model: DV-III ULTRA, Brookfield Engineering Lab; Inc; Middleboro, USA) fitted with a Cp-52 cone spindle. pH was determined using pH meter (Model: 7007, Digisun electronics, Hyderabad, India).

Spreadability
Apparatus required for the study of spreadability of formulated gels was fabricated. It consists of a wooden block, which was provided by a pulley at one end. A ground glass slide was fixed on the block and an excess of formulated gel (2 g) was placed on it. Gel was sandwiched by using another glass slide with dimensions as that of fixed ground slide and provided with hook. Weight (1 kg) was placed on the top of two slides for few minutes to remove entrapped air and to form a uniform gel film between slides. Excess of gel from the edges was scraped off. Pulley was attached to the hook and weight was incorporated to it. Time taken by top slide to travel a distance of 7.5 cm was noted.

In vitro Drug Diffusion Studies
Drug diffusion rate from different gel formulations were studied by modified Keishery Chein Cell using cellophane membrane (MWCO 12-14 kD, HIMEDIA, Mumbai, India) as a barrier. Diffusion cell was assembled on magnetic stirrer along with diffusion membrane (effective surface area, 3.8 cm$^2$), which separates donor and receptor compartments. Diffusion membrane was immersed in receptor compartment having aqueous solution of 1% w/v SLS as diffusion medium, maintained at 32±2°C for 30 min for equilibrium. Gel (1 g) was kept on membrane in donor compartment. At predetermined intervals, medium (5 ml) was withdrawn from receptor compartment for 6 h. Withdrawn sample was replaced by the same fresh medium. Absorbance of these samples was measured spectrophotometrically at 239 nm by UV-VIS double beam spectrophotometer (Model: Shimadzu-1700, Shimadzu Corp., Japan). Cumulative release (%) of valdecoxib from different gel formulations was calculated.

Skin Compatibility Studies
Protocols of this study were approved by Institutional Animal Ethical Committee of NET Pharmacy College, Raichur, India. Male albino rats (av body wt 200-250 g) were divided into 3 groups having 5 animals in each group. Group I was served as control while Group II and III were treated as test groups. Hairs on ventral surface were depleted and gel formulations were applied twice a day for 1 week. Animals were supervised for allergic manifestations, if any.

Stability Studies
This study was performed as per the reported method. All formulations were exposed in a cyclic pattern to different temperatures (−5°C to 25°C for 5 cycles) at every 24 h. Gels were characterized for physical stability and syneresis. As per ICH guidelines, 3 months stability studies were also done by subjecting gels to different temperatures (25±2°C to 40±2°C) and relative humidity (75±5% RH). In this regard, drug
content, viscosity, pH and in vitro diffusion data were collected at every month.

**Cyclooxygenase-2 Inhibition Study**

COX-2 enzyme is involved in production of PGs, which causes inflammation. Hence, this study was performed to estimate anti-inflammatory activity of formulated gels. In this regard, albino rats (200-250 g) were divided into 2 groups of 6 animals each. Oedema was induced in hind paw of rats by injecting 1% w/v carrageenan (0.1 ml) into plantar surface and paw volume was measured by dipping in mercury column of plethysmometer. Optimized gel formulation was applied with slight rubbing. Paw oedema was directly compared with change in height of mercury produced by the control and test group animals. Paw volume was measured at every 1 h up to 6 h for 7 days. Control group rats received only gel base without drug by same mode of application. Inhibition (%) of oedema = (V_c – V_t / V_c) x 100, where V_c is av. paw volume in control group and V_t is av. paw volume in drug treated group.

**In vitro 5-Lipoxygenase Inhibition Study**

Using in-vitro assay method, 5-lipoxygenase inhibitory potential of optimized valdecoxib gel formulation was determined. Mixture of linoleic acid (80 mM) and potato 5-lipoxygenase (10 µl) in phosphate buffer (50 mM, pH 6.3) was treated as blank and enzyme activity was measured at λ_max 234 nm. Enzyme inhibition by valdecoxib gel was measured spectrophotometrically by incubating gel (0.1, 0.2, 0.3 0.4 and 0.5 g) with above mixture for 5 min. Filtered at each time to remove particulate matters, if any. Inhibition (%) was calculated by comparing slope of gel formulations with that of enzyme activity.

**Results and Discussion**

Valdecoxib topical gels were formulated by using various gelling agents (MC, Carbopol 934 and HPMC) to provide adequate consistency and elegancy. In order to solubilize drug in the formulation, PG was used as a solvent as well as humectants to avoid drying of gels. Hydroalcoholic vehicle (2:1), which enhances hydration of gelling agents, is essential as a structure forming and stabilizing agent. It also plays a crucial role in gellation. Alcohol also acts as co-solvent for the drug. Alcohol (high conc.) reduces viscosity of formulated gels, may be due to break down of cross linking between the polymer. Being dead and horny, stratum corneum of skin acts as a main barrier for topical delivery of drug. Menthol alters properties of this barrier, so enhances drug delivery through skin by increasing both the concentration and diffusion rate in skin. Menthol contains functional group of hydrogen bonding and it is a lipophillic terpene found to be more effective because it enhances penetration of drug by both lipid and pore pathway. Menthol provides cooling sensation after applying formulation to skin. Methyl and propyl paraben are well known preservatives used to avoid microbial growth. Different gel formulations were developed by using MC (A1, A2 and A3), Carbopol 934 (B1, B2 and B3) and HPMC (C1, C2 and C3) in various concentrations (0.5, 1 and 2 g) to optimize gel formulation on the basis of its consistency and in vitro drug release. (Table 1). Formulated gels were found free from clogs and sudden viscosity changes. Post application feel of all gels were smooth and comfortable. Valdecoxib gels with MC and Carboprol 934 were transparent, whereas HPMC gel was whitish, may be due high cross linking property of HPMC.

Drug content of formulated gels was found from 98.49 ± 0.64 to 99.95 ± 0.63% (Table 2), indicating that there is no significant variation. Since viscosity is inversely proportional to in vitro drug release, hence a change in viscosity reduces effectiveness of the product. Viscosity of formulations depends upon concentration, molecular weight and degree of cross linking of polymers.
high as compared to that of MC and HPMC gels (Table 2). It is because of higher molecular weight of Carbopol 934 and it high degree of cross linking, which immobilize liquid to flow out. All formulated gels had pH from 6.82 ± 0.14 to 7.02 ± 0.10 (Table 2). It was found that viscosities of all gels were in accordance with skin pH, thus it minimizes the chances of skin irritation. In order to give desired therapeutic effect to affected area, spreadability of gel is essential to that particular local site. Spreadability depends upon viscosity of gels (18.43 ± 0.14 to 37.94 ± 0.09 gcm/s for all formulations (Table 2). Comparative data of cumulative (%) drug release for 6 h study from various gels (Table 2) indicated that in vitro drug release inversely depends upon polymer concentration. Water and PG hydrate stratum corneum to open the channels, through which drug can pass in the dipper of skin layers. Alcohol evaporation on skin surface increases drug fraction on skin, which creates concentration gradient that is driving force for drug penetration. Also, alcohol fluidizes skin surface to enhance drug permeation. But concentration of alcohol used to be in limited fraction because excess of alcohol harms skin. High rates of drug release were found in Carbopol 934 gels as compared to gels formulated with MC and HPMC (Fig. 2). Kinetics of drug release from gels (Table 2) revealed that all gels followed zero order kinetics as their R² values ranges between 0.9869 to 0.9986 and mechanism of drug release was found to be diffusion controlled (Higuchi data), which is the rate the rate limiting step in drug permeation. Among all batches, formulation B1 (0.5 g, Carbopol 934) passed all essential criteria as an optimized formulation (Table 2) with desirable in vitro release (15.48 ± 0.06%), viscosity (2243.9 ± 0.04 cpc) and spreadability (27.53 ±0.06 gcm/s).

Table 2—Evaluation parameters of valdecoxib gels [values are represented as Mean±SD, (n=3)]

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content %</th>
<th>Viscosity cps</th>
<th>pH</th>
<th>Spreadability gcm/s</th>
<th>Drug release in 6 h, % Q</th>
<th>Cumulative drug release in 6 h, % Q/A</th>
<th>First order R²</th>
<th>Zero order R²</th>
<th>Higuchi model R²</th>
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<tr>
<td>A1</td>
<td>99.81±0.36</td>
<td>1156.2±0.08</td>
<td>6.99±0.14</td>
<td>18.43±0.14</td>
<td>53.21±0.04</td>
<td>14.01±0.03</td>
<td>0.9763</td>
<td>0.9963</td>
<td>0.9686</td>
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<tr>
<td>A2</td>
<td>99.62±0.32</td>
<td>2983.4±0.03</td>
<td>6.91±0.02</td>
<td>31.47±0.13</td>
<td>41.39±0.13</td>
<td>10.89±0.05</td>
<td>0.9876</td>
<td>0.9965</td>
<td>0.9674</td>
</tr>
<tr>
<td>A3</td>
<td>98.49±0.64</td>
<td>4136.3±0.06</td>
<td>6.93±0.19</td>
<td>36.02±0.03</td>
<td>32.92±0.21</td>
<td>8.66±0.02</td>
<td>0.9871</td>
<td>0.9952</td>
<td>0.9749</td>
</tr>
<tr>
<td>B1</td>
<td>99.95±0.63</td>
<td>2243.9±0.04</td>
<td>7.02±0.10</td>
<td>27.53±0.06</td>
<td>58.83±0.09</td>
<td>15.48±0.06</td>
<td>0.9710</td>
<td>0.9979</td>
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<tr>
<td>B2</td>
<td>98.76±0.43</td>
<td>3254.4±0.01</td>
<td>6.93±0.03</td>
<td>34.68±0.43</td>
<td>34.83±0.03</td>
<td>9.16±0.04</td>
<td>0.9833</td>
<td>0.9938</td>
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<td>B3</td>
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<td>4634.3±0.13</td>
<td>6.96±0.06</td>
<td>37.94±0.09</td>
<td>28.32±0.24</td>
<td>7.45±0.05</td>
<td>0.9765</td>
<td>0.9869</td>
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<td>C1</td>
<td>99.59±0.54</td>
<td>1364.4±0.04</td>
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<td>19.88±0.12</td>
<td>50.46±0.73</td>
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<tr>
<td>C2</td>
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<td>1923.5±0.08</td>
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<td>22.42±0.42</td>
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<tr>
<td>C3</td>
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<td>7.00±0.02</td>
<td>29.75±0.04</td>
<td>43.67±0.54</td>
<td>11.49±0.01</td>
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Fig. 2—In vitro drug release profile of valdecoxib from: a) MC gels; b) carbopol gels; and c) HPMC gels
All gels are composed of pharmaceutically approved (non-immunogenic and biocompatible) excipients in desired amounts. But still there may be chances of some allergic manifestations after applying on skin. Hence, skin compatibility study showed some behavioral changes in rats after first application, may be due to cooling effect by menthol and alcohol. But on further application, they showed tolerability to that action. No allergic manifestation was observed during 7 days study. Under stability study, when formulations were exposed to various temperature conditions in cyclic pattern (25 and –5°C), no changes were found in their physical stability and did not showed syneresis. Also, all formulations passed stability tests as per ICH guidelines for three months. No significant changes were found in drug content, viscosity, pH and in vitro drug release (Table 3), indicating that formulated gels were stable.

Anti-inflammatory potential of optimized gel formulation (B1) showed (Table 4) that maximum inhibition of rat paw oedema was up to 79.51%, which is in accordance with reported value. It showed that optimized formulation (B1) had significant topical potential to inhibit oedema. In vitro 5-lipoxygenase inhibition by optimized valdecoxib gel was carried with increasing dosage manner. But IC$_{50}$ value for 5-LOX inhibition was not significant (Fig. 3) even though higher dosage of B1 were treated.

**Conclusions**

Oral delivery of valdecoxib causes life threatening gastric bleeding and other side effects but topical gel encounters all side effects associated with oral delivery. Among all gels of valdecoxib developed by using methyl cellulose, carbopol and HPMC, carbopol gel was found
to be very effective against COX-2. In future it may be promising and attentive against local inflammation.

References