DEVELOPMENT OF ECONOMIC TRANSDERMAL PATCHES CONTAINING LORNOXICAM FOR TREATMENT OF ACUTE AND CHRONIC INFLAMMATORY MODELS IN ALBINO RATS (A PRECLINICAL STUDY)

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Keywords:
Lornoxicam, Transdermal patch, Anti-inflammatory activity, Rat paw edema, Granuloma pouch

ABSTRACT:
Objective: To formulate economic transdermal patch of lornoxicam (LX) and to study its anti-inflammatory activity on acute and chronic inflammatory in rat models
Method: Three LX transdermal patches (F1, F2 and F3) consist of Eudragit RL-100 and RS-100 in three different ratios (1:2, 1:1 and 2:1) were prepared respectively by the solvent evaporation technique. All formulations contain diethylphthalate as plasticizer. The physicochemical compatibility of the drug and the polymers was studied by differential scanning calorimetry. The prepared LX transdermal patches were evaluated for their drug content, patch thickness, folding endurance, surface pH and in vitro drug permeation. The anti-inflammatory activity of LX transdermal patch was determined using two animal experimental designs one for acute inflammation (rat paw edema) and the other for chronic inflammation (granuloma pouch) on eighty adult albino rats. The results obtained were statistically analyzed.
Results: The data showed physicochemical compatibility between the drug and the polymers used. The LX patches (F1, F2 and F3) prepared were uniform thickness, drug content and the pH of LX patch was found to be 6.5. Increasing the ratio of the Eudragit® RL-100 polymer to eudragit RS 100 (F3) showed a corresponding increase in the folding endurance of patches. Diethyl phthalate led to increase elasticity for the patches. F3 showed higher drug permeation than F2 and F1. Edema (anti-inflammatory effect) was significantly decreased in group V followed by group IV then by group III in case of the two animal experimental designs. Group V received LX transdermal patch (F3).
Conclusion: The present study showed that the prepared economic lornoxicam transdermal patch has good properties with a high efficiency as an anti-inflammatory drug on acute and chronic inflammatory models.

INTRODUCTION: Inflammation is a reaction of tissue to injury, expressed by five cardinal points: pain, swelling, redness, heat, and loss of function.

Inflammation and pain relieving drugs are the most commonly used drugs worldwide either through prescription or as over the counter medication.
None steroidal anti-inflammatory drugs (NSAIDs) have analgesic, antipyretic, and anti-inflammatory effects. Lornoxicam (LX) is a new NSAID belonging to the oxicam class. As with other NSAIDs, the principle mechanism of action of LX relates to the inhibition of cyclooxygenase (COX), the key enzyme of the arachidonic acid pathway, resulting in the inhibition of prostaglandin (PG) synthesis. It differs from other oxicam compounds in its potent inhibition of (PGs) biosynthesis, a property that explains the pronounced efficacy of the drug. (PGs) are involved in all phases of inflammatory events including fever, pain reaction and physiological functions like intestinal motility, vascular tone and gastric acid secretion. LX is distinguished from established oxics by its short plasma half-life (3-5h) and good gastro-intestinal tolerability.

Thus, in rat polymorphonuclear leukocytes, LX potently inhibited the formation of PGD2 in vitro. In vivo LX exhibited anti-inflammatory activities in acute (carrageenan-induced paw oedema) as well as in chronic inflammation (adjuvant induced arthritis) that were more potently than the ones of other oxicams. Clinical investigations have established LX as a potent analgesic with excellent anti-inflammatory properties in a range of painful or inflammatory conditions, including postoperative pain and rumatoid arthritis (RA). LX had protective effects on myocardial infarction in rats under ischemia and ischemia reperfusion.

An experimental study in mice has demonstrated its protective effects against herpetic stomatitis (HSK) through down-regulation of nuclear factor Kappa B (NF-Kappa B) activation. It is effectively relieved the symptoms of osteoarthritis, and ankylosing spondylitis. LX had shown strong analgesic effects after dental surgery, hysterectomy, Lumbar disk surgery and in controlling lower back pain. In a series of studies, LX was administered intraperitoneally, intravenously, intramuscularly and this play a role in mechanism of systemic absorption and incidence of adverse effects. Studies the effect of the drug by topical application are not enough studies. Thus, the purpose of this study is to develop economic LX transdermal patch to investigate LX effects as anti-inflammatory for treatment of inflammation in rat model in acute and chronic models.

MATERIALS AND METHODS:

Materials: Lornoxicam (LX) was kindly donated by Delta Pharmaceuticals company, Cairo, Egypt; Eudragit® RS-100 and RL-100 were a gift from Evonic, Darmstadt, Germany; Diethylphthalate (Lobochemie Pvt. Ltd, India), methyl alcohol, ethyl alcohol, calcium chloride, sodium chloride, sodium hydrogen phosphate and potassium dihydrogen phosphate of analytical grade were purchased from Adwic pharmaceutical company, Egypt. Carrageenan was purchased from Sigma-Aldrich Chemicals Co., ST Louis USA.

Methods:

Preparation of Lornoxicam (LX) transdermal patches: LX transdermal patches contained 16mg lornoxicam were prepared using Eudragit® RL-100 and RS-100 in three different ratios: 1:2 (F1), 1:1 (F2) and 2:1 (F3) respectively. Diethyl phthalate was used as plasticizer in concentration of 20% w/w. The transdermal patches were prepared by solvent evaporation casting technique as follows: Eudragit polymers, drug and plasticizer were dissolved in methanol. The alcoholic solution was mixed in a beaker and stirred with magnetic stirrer to accomplish a homogeneous mixture and the resulting solution was poured in a plastic container of diameter 2cm. The solvent was allowed to evaporate at ambient conditions and the obtained transdermal patches were stored in a desiccator over calcium chloride (CaCl2) and evaluated within one week.

Evaluation of lornoxicam transdermal patches:

1. Drug content of the patch: A 2cm² patch was cut into small pieces, put into a 100 ml phosphate buffered saline PBS (pH 7.4), and shaken in a mechanical shaker at 37°C and 100rpm for 24h. Then the whole solution was ultrasonicated for 15min. After filtration, the drug was estimated spectrophotometrically (UV-VS-Spectrophotometer, multicell changer, 6705-Jenway, Bibby scientific, Ltd, UK) at wavelength of 376 nm and the concentration...
was deduced in accordance to a pre-constructed calibration curve in PBS (pH 7.4). The experiment was repeated three times and the mean drug content for each formulation was determined.

2. **LX patch thickness:** The thickness of the patches was determined by measuring the thickness at the 4 edges and the center of the formulated films using a micrometer screw gauge (Mitutoyo Corp, Japan) and the results were expressed as average thickness ± SD.

3. **Differential scanning calorimetry (DSC):** Samples of drug, pure patch components and the physical mixture of the drug and the polymers were analyzed by DSC using a Shimadzu DSC-60 (Shimadzu, Kyoto, Japan).

4. **Folding endurance of LX patches:** The mechanical properties of the transdermal patches were determined. Three patches of each formulation of size 2x2cm were cut by using sharp blade. Folding endurance of the buccal films was determined by repeatedly folding patch at the same place till it broke. The mean value was calculated.

5. **Determination of LX patch surface pH:** Drug patch was left to swell for 30 minutes on the surface of 2% w/v agar plate. The surface pH was measured by means of pH paper (Whatman, USA) placed on the surface of the swollen patch.

6. **In-vitro drug permeation from patch:** The drug permeated from different transdermal patches through synthetic cellulose acetate membrane was studied. USP dissolution tester (BLHMO15K-10, Oriental Motor, Japan) of basket type with a slight modification was utilized. Glass cylinders of 10cm length and 2.5cm diameter were used instead of the regular baskets. Cellulose acetate membrane with a 12,000-14,000 molecular weight cut off was soaked in saline PBS (pH 7.4) overnight before the study.

Animal and Experimental design: This study was conducted on eighty adult albino rats weighing 180-200gm. They bred in the animal house of Kasr El Aini, Faculty of Medicine, Cairo University. They were caged in fully ventilated room, exposed to natural daily light/dark cycle. They were allowed food and water ad libitum. The animals were handled according to the guidelines of Helsinki declaration rights (1975) of using laboratory animals.

1. **For Acute Experimental (Rat paw edema):** Animals were divided into 5 groups each of 8 animals. Carrageenan induced rat paw edema in all groups for the present study according to the method described by Winter and El Nabarawi et al.

Suspension of 1% carrageenan is prepared freshly in normal saline and injected into subplantar region of right hind paw in a dose 0.1 ml. The intensity of edema was assessed by measuring the paw volume (ml) by plethysmometer (7410 Ugo Basile, Italy) after 1 hour and 5 hours. The edema and inhibition rate percentage of each group was calculated as follows:

Equation 1: Determination of edema rate %

Edema rate (E%) =
Equation 2: Determination of Inhibition rate %

\[
\text{Inhibition rate} = \left( \frac{V_0 - V_t}{V_0} \right) \times 100
\]

Where \(V_0\) is the mean paw volume before carrageenan injection (ml), \(V_t\) is the mean paw volume after carrageenan injection (ml), \(E_c\) is the edema rate of control group and \(E_t\) is the edema rate of the treated group.

Group I received no treatment, Group II rats received LX 5mg/kg parenterally SC (behind the neck) one hour before induction of inflammation. Group III, Group IV and Group V were subjected to application of LX patch (F1), (F2), (F3) respectively.

2. For Chronic experimental (Granuloma pouch 25): Forty white albino rats were divided into five groups each containing 8 rats. The hair of each rat was removed with electric hair clipper from the skin of the dorsal region 48 hours prior to use. The shaved area was investigated that’s no wounds or any blood comes out. Sterile air (10ml) was injected into the loose SC connective tissue of each rat back. Carrageenan gel solution (2%), 5ml was then injected into this air mould. The rats were then left for one day later; the air bleb was deflated to accelerate the formation of the exudate.

The animals (40 rats) were divided according to the scheme described below: All groups received Carrageenan gel solution in the dorsal back of each rat. Group I received no treatment Group II, III, IV, V received treatment as previously described in acute experiment model. Before application of LX patches to the dorsal of rats, a piece of cotton wetted with ethanol was used to clean the skin from any debris three times respectively. LX patches were applied to the dorsal shaved area and covered with a piece of adhesive tape.

Each rat in each group was put in separate cage and left for 7 days. The rats were under observation all over 7 days after application. At the end of 7 days, the rats were slaughtered and the volume of exudate present in the air cavity was determined for each rat in each group using small pipette. The volume of exudate and percent inhibition in volume for all groups was measured.

Statistical analysis methods: Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically. Qualitative variables were expressed as percentages. Quantitative variables from normal distribution were expressed as mean and S.D. One Way ANOVA used to declare the significant difference between groups at \(p < 0.05\). Duncan multiple comparison test at \(p < 0.05\) was used to declare the significant between each group and the control group.

RESULTS AND DISCUSSION: A transdermal patches of lornoxicam were prepared with Eudragit RL 100: Eudragit RS 100 in three different ratios with diethylphthalate as plasticizer using the solvent evaporation technique. Results showed that the drug content of the patches was ranged from 96.45 to 103.33%. The patches thickness was ranged from 0.36 to 0.46 mm. Results obtained showed that films prepared were uniform thickness. The difference in thickness was due the concentration of polymer in each device. The pH of LX patch was found to be 6.5.

Figure 1 showed the thermograms of pure drug and the physical mixture of the drug and the polymers (Eudragit® RL-100, Eudragit® RS-100) in ratio 1:1. The superimposition of the thermograms of the physical mixture, prepared by simple blending of drug and polymer, to those of the patch components indicated the absence of interactions between the drug and the polymers.

The obtained data revealed that, when increasing the ratio of the Eudragit® RL-100 polymer (F3) there was a corresponding increase in the folding endurance of patches. Diethyl phthalate 20% in the Eudragit® patches led to increase elasticity for the patches. The order of increasing folding endurance
of patches was in order: F3 > F2 > F1. These results were in a good agreement of Entwistle C and Rowe R. They found that DEP in the Eudragit® film increase in elongation and correspond in decrease in tensile strength. This was due to the fact that plasticizers acted by inserting themselves between the polymer strands, breaking the polymer-polymer bond which led to an increase the molecular mobility of the polymer strand.

Thus, it was expected that as the concentration of the plasticizer increased, the degree of the film stiffness decreased whereas the film ductility increased. Upon addition of plasticizer, flexibilities of polymer macromolecules or macromolecular segments increase as a result of loosening of tightness of intermolecular forces. It was also found that weakening of interaction of the polymer chains led to a decrease in the tensile strength and an increase in the percent elongation of the films.

Figure 2 showed the permeation profile of lornoxicam patches (F1, F2 and F3) composed of different ratios of Eudragit® RL-100: RS-100 in PBS (pH 7.4). The permeation of drug from F3 showed higher drug release than F2 and F1 this could be explained on the fact that, F3 which was composed of higher ratio of Eudragit® RL-100. These results could be attributed to the lower content of quaternary ammonium groups present in Eudragit® RL-100 than in RS-100, resulting in less swelling in the aqueous medium.

In addition, the inclusion of Eudragit® RS-100 polymer in the films led to a slight reduction in the permeation profile of the drug F2 and F1. In case of the patches prepared using higher percent of Eudragit® RS-100 (F1 and F2), it was found that the drug permeation decreased significantly.
Table 2 showed the maximum edema was shown group I. Edema was significantly decrease in group V followed by group IV followed by group III and then group II. These results demonstrated that the maximum inhibition of edema was by patch F3 and not by injection as in acute study. Our results are in accordance with 31, 32 which stated that LX is approved for intravenous, intramuscular and oral use, no toxicological data are available regarding its intra-articular administration. The peripheral effects of NSAIDs are peripheral, and therefore, local application to the site of injury should produce analgesia while minimizing systemic side effects.

**TABLE 1: EFFECT OF LX PATCHES TESTED ON RAT PAW EDEMA AFTER ONE AND FIVE HOURS.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Formulation</th>
<th>% Swelling 1 h</th>
<th>% Swelling 5 h</th>
<th>Z-value</th>
<th>p-value</th>
<th>% Inhibition 1 h</th>
<th>% Inhibition 5 h</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>32</td>
<td>59</td>
<td>3.98</td>
<td>0.000*</td>
<td>68</td>
<td>41</td>
<td>3.98</td>
<td>0.000*</td>
</tr>
<tr>
<td>II</td>
<td>Injection</td>
<td>18</td>
<td>29</td>
<td>1.85</td>
<td>0.069</td>
<td>82</td>
<td>71</td>
<td>1.85</td>
<td>0.064</td>
</tr>
<tr>
<td>III</td>
<td>F1</td>
<td>30</td>
<td>26</td>
<td>0.63</td>
<td>0.528</td>
<td>70</td>
<td>74</td>
<td>0.63</td>
<td>0.528</td>
</tr>
<tr>
<td>IV</td>
<td>F2</td>
<td>29</td>
<td>20</td>
<td>1.49</td>
<td>0.137</td>
<td>71</td>
<td>80</td>
<td>1.49</td>
<td>0.137</td>
</tr>
<tr>
<td>V</td>
<td>F3</td>
<td>27</td>
<td>15</td>
<td>2.11</td>
<td>0.035*</td>
<td>73</td>
<td>85</td>
<td>2.11</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

* = There is a significant difference between the two proportions by using Fisher’s exact test at p<0.05

**TABLE 2: EFFECT OF MEDICATED F1, F2, F3 AND S.C LORNOXICAM IN REDUCING VOLUME OF INFLAMMATORY EXUDATES AFTER 7 DAYS**

<table>
<thead>
<tr>
<th>Time</th>
<th>Formulation</th>
<th>Mean ± S.D</th>
<th>% ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td>Carrageenan gel solution</td>
<td>2.76 ± 0.06 e</td>
<td>7.64 ± 0.01 a</td>
</tr>
<tr>
<td></td>
<td>Commercial injection</td>
<td>2.55 ± 0.03 d</td>
<td>19.63 ± 0.01 b</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>1.94 ± 0.06 b</td>
<td>29.82 ± 0.04 c</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>2.22 ± 0.07 c</td>
<td>30.66 ± 0.05 a</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>1.35 ± 0.05 a</td>
<td>51.26 ± 0.05 d</td>
</tr>
<tr>
<td></td>
<td>F-value</td>
<td>300.662</td>
<td>1062469.6</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

While COX 1 is thought to account for homeostatic amounts of eicosanoids, COX2 is induced during inflammation leading to the formation of pathologic amounts of prostaglandins (PGS). The inhibition of (PGS) synthesis by NSAIDs has been demonstrated to reduce inflammatory symptoms such as edema and pain 34, 35, 36.

Other mediators of inflammation such as reactive oxygen products and cytokines which contribute to inflammation and pain 37, 38 leading to transcriptional induction of COX-2 gene, the expression of the gene encoding inducible nitric oxide synthase (iNos) is induced, leading to increase level of nitric oxide in inflammation tissue which leads to edema formation and pain 38, 39.

LX was found to be the most potent balanced inhibitor of human COX 1, 2. The in vitro activities described support the marked anti-inflammatory and analgesic activities of LX found in animal models as well as in clinical studies 37.

Smith et al 33 showed that the principal mechanism of action of NSAIDs relies on inhibit up of COX which is the rate limiting enzyme in the arachidonic acid pathway. COX exists in two isoforms COX 1 and COX 2.
LX is at least 10 times more potent as anti-inflammatory agent than piroxicam and was also tenfold more active than tenoxicam in inhibiting carrageenan-induced edema in the rat paw swelling in the adjuvant induced polyarthritic rat 8. 11.

The volume of exudates obtained was significantly decreased by F3 followed by F2 followed by F1 after 7 days treatment. These results were in a good agreement with in vitro drug permeation from different patches through cellulose acetate membrane. The order of decreasing volume of exudates was as follows: F3 > F2 > F1 > injection.

The potent nonselective COX inhibition by LX was observed in vitro is in good agreement with animal experiments where inhibition of COX 1 mediated PGS synthesis is thought to be involved in compound efficacy such as acetyl choline-induced writhing or where COX 2 inhibition such as carrageenan-induced paw edema has been suggested to be causal for anti-inflammatory effects 36. Furthermore, LX was potently effective as anti-inflammatory and analgesic activity 14-16.

Moreover a number of studies have examined the role of nonselective COX inhibitors both in animal models and in patients 40, 41, 42 demonstrated beneficial effects of NSAIDs on the TNF-α level which can be ameliorated 43.

LX has also shown marked inhibitory activity on endotoxin induced IL6 formation in THP1 monocytes with less activity on TNF-α and IL-1. LX treatment suppressed both NF-Kappa B activation and TNF-α expression in biological analysis 44, 45.

In addition Pruss et al 5 stated that the powerful inhibition of cyclo-oxygenase has manifested itself as highly potent analgesic and anti-inflammatory effects in animal studies. Studies have demonstrated that LX inhibits polymorphonuclear (PMN) leukocyte migration, inhibits release of superoxide from human PMN-leukocytes, inhibits release of platelet derived growth factor (PDGF) from human platelets and stimulate the synthesis of proteoglycans in cartilage in tissue culture 8, 37.

CONCLUSION: The present study showed that lornoxicam transdermal patches proved to be economic in manufacture, non-irritant and had anti-inflammatory effects on acute and chronic models of inflammation using albino rats. In addition, topical application of LX transdermal patch reduces side effects of drug which induced by other routes. Furthermore, F3 patch possesses superior to injection route in treatment of edema in chronic models.

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