SUSTAINED ANTI-INFLAMMATORY EFFECT OF TOLMETIN SODIUM IN TRANSDERMAL PATCHES

HASSAN M. ELSABBAGH, GALAL M. ABDELGHANI, AYA A. NASHAAT

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

Accepted Date: 21/02/2016; Published Date: 27/02/2016

Abstract: Tolmetin sodium is a non-steroidal anti-inflammatory drug. NSAIDs are known for their adverse effects on the gastrointestinal tract. The objective of this study is to formulate Tolmetin sodium into transdermal drug delivery system (TDS) to avoid the GIT adverse effects. Different polymers; Eudragit E 100, Eudragit L 100-55 and hydroxypropyl methylcellulose (HPMC), each is used with different concentrations; (4%, 6%, 8% and 10%) in the formulation of monolithic transdermal patches. The in vitro release of the drug from the formulated patches was tested through dialysis membrane (cellulose membrane). The transdermal patches containing 4% Eudragit L 100-55 gave highest in vitro release profiles and was chosen for in vivo study. The anti-inflammatory activity of this formulation was evaluated using carrageenan induced rat paw oedema model, which revealed a significant sustained anti-inflammatory activity.

Keywords: Tolmetin sodium, transdermal patches, Eudragit E 100, Eudragit L 100-55, hydroxypropyl methylcellulose, carrageenan.

Corresponding Author: PROF. H M ELSABBAGH

Access Online On:
www.ijprbs.com

How to Cite This Article:
HM Elsabbagh, IJPRBS, 2016; Volume 5(1): 136-151
1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs for the management of acute and chronic inflammatory processes\[^1\-2\]. Most NSAIDs are considered safe but their oral administration may cause several side effects in patients\[^3\-4\]. The most frequently reported side effects of NSAIDs are gastrointestinal diseases with liver and kidney toxicity. Therefore, there is a growing interest in the development of alternative routes of administration for these drugs\[^3\-5\]. The route of choice is the transdermal one in which the drug agent is applied to the skin so that sufficient quantity penetrates the skin to exert either a local or a systemic effect.

A transdermal patch is a medicated adhesive patch that is applied to the skin to deliver a specific dose of medication through the skin and into the bloodstream to exert either a local or a systemic effect. The first transdermal system for systemic delivery was approved for use in the United States in 1979. It was a three-day patch that delivers scopolamine to treat motion sickness\[^6\].

The carrageenan induced inflammation, originally described by Winter CA et al. 1962\[^7\], is an acute, non-immune, well-researched, and highly reproducible model for assessing anti-inflammatory activity. Inhibition of the induced inflammation has been shown to be highly predictive and doses of NSAIDs in this model correlate well with the effective dose in patients\[^8\]. The use of antagonists of various mediators of inflammation revealed that the inflammatory response to carrageenan consisted of three phases\[^9\]. The primary phase mediated by both histamine and 5-hydroxytryptamine. The secondary phase is kinin-mediated and the final phase is caused by the local production of prostaglandins (PG), especially those of the E series.

Carrageenans are a complex group of polysaccharides made up of repeated galactose-related monomers and are of three main types; lambda, kappa, and iota. Each type has different gel characteristics. The lambda form does not gel strongly at room temperature and could be injected to induce an inflammatory response\[^10\].

2. Materials and methods:

2.1. Materials:

- Tolmetin sodium was purchased from sigma Aldrich (Germany).

- Hydroxypropyl methylcellulose (HPMC), Alfa Aesar (Germany).
• Eudragit L 100-55 was received as gift sample from Sigma (Egypt).

• Eudragit E 100, Rohm Pharm GMPH. , Pharmaceutical Laboratory (Germany).

• Spectra/Por dialysis membrane, 12,000ñ14,000 molecular weight cut off (Spectrum Laboratories Inc., Rancho Dominguez, Canada).

• Carrageenan, Sigma (St. Louis, MO, USA).

• Normal Saline (0.9 % sodium chloride).

• Adhesive tape (Silkplast), pharmaplast, Egypt.

All solvents and reagents used were of analytical reagent grade.

2.2. Equipment:

• PH meter, Beckman Instrument Fullerton, CA 92634, Germany.

• Electric balance, Zakiady Mechanikr Precyzyjnej Merrwag Gdansk, Poland.

• Ultra violet-visible spectrophotometer, JASCO, V-530, Japan.

• Abbotoa, Abbota corporation dissolution apparatus.

• Elora digital caliber (150 mm), Germany.

• Insulin disposable syringes.

2.3. Standard Curve for Tolmetin sodium:

A stock solution of Tolmetin sodium was prepared by dissolving 10 mg in 100 ml phosphate buffered saline; PBS (pH 7.4) in a volumetric flask.

The stock solution of Tolmetin sodium was subsequently diluted with PBS (pH 7.4) to obtain a series of concentrations; 4, 6, 8, 10 and 12 μg of Tolmetin sodium per ml of solution.
The maximum absorbance of Tolmetin sodium was at 323nm. Absorbance of the above dilutions was measured in double beam UV spectrophotometer at 323 nm using PBS (pH 7.4) as a blank. Reproducibility of the method was tested by analysing three separately weighed samples of Tolmetin sodium.

2.4. Preparation of Tolmetin sodium Transdermal Patches:

2.4.1. Eudragit Patches:

Monolithic design of transdermal patches of Tolmetin sodium was prepared using different concentrations of Eudragit E 100 and Eudragit L 100-55 polymeric solutions. Four solutions of each polymer were prepared with different concentrations 4%, 6%, 8% and 10% (w/v) using 2% di-n-butyl phthalate as a plasticiser\(^{[11]}\). Silk fabric was used as the backing layer of the patches.

The drug was dissolved in polymeric solution, and then poured on the backing layer. The patches were then left to dry at room temperature for 24 hours.

After drying the patches were wrapped with aluminium foil until they were used for further study.

2.4.2. HPMC Patches:

The release results of patches containing 6% HPMC through cellulose membrane were found to be statistically non-significant (p>0.05) compared with concentration of 4% HPMC. Based on these results a higher concentration formulation containing 12% (w/v) HPMC was added to the in vitro release study. Monolithic type transdermal patches of Tolmetin sodium were prepared as mentioned before using 2% glycerol as a plasticiser.

2.5. In-vitro evaluation of transdermal patches:

In-vitro permeation studies were carried out using dissolution apparatus. Every patch was sealed inside dialysis membrane and tied firmly to a paddle. The receptor media was 900 ml PBS with pH 7.4 equilibrated to 37 ± 0.5 °C. The content of each cell was stirred at a constant speed (100 rpm). Samples were withdrawn at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 hours intervals. Each withdrawn sample is replaced with equivalent volume of phosphate buffer at the same temperature to keep the volume constant. The samples were analysed for drug content using UV spectrophotometer at λ max 323 nm against the blank. The permeation study was carried out for 6 hours and repeated three times for each polymer concentration and the average was calculated.
2.6. In Vivo Evaluation of Tolmetin Sodium - Eudragit L 100-55 Transdermal Patches:

2.6.1. Animals:

Male albino rats of approximately the same age weighing 310-350 gm were obtained from the Experimental Animal House, Faculty of Pharmacy, Mansoura University. The animals were kept under standard laboratory conditions with free access to a standard laboratory diet and water.

2.6.2. In vivo study:

The anti-inflammatory activity of the formulation was evaluated by the carrageenan-induced hind paw oedema method.

Animals were weighed, randomised into two groups (n=5). The animals were identified by marking the tail with ink. The thickness of the paws was measured before giving carrageenan injection. Paw volumes were measured at hourly intervals for 6 hours and again after 24 hours [10].

The induction of inflammation in the rats was made by injecting 0.1 ml of 1% solution of carrageenan in 0.9% saline subcutaneously into the plantar region of the left hind paw using disposable insulin syringes [7]. Transdermal patches containing 4% Eudragit L 100-55 were applied on the shaved area of the dorsal region after thirty minutes of carrageenan injection. The results were expressed as the percentage increase of paw thickness at each time interval [12].

The dorsal regions of the rats were shaved 24 hours before starting the experiment. The animals were then divided into two groups of five animals each.

Group 1: Drug free transdermal patches were applied (Control group).

Group 2: Medicated transdermal patches were applied (Treated group).

Tolmetin sodium transdermal patches containing 4% Eudragit L 100-55 were applied on the shaved area of the dorsal region of the animals using adhesive tape. Measurements of paw thickness were done using a digital caliper (mm), to the precision of two decimal numbers.

The percentage increase of paw thickness from time zero was calculated for each group using the following equation:
Percentage Oedema (%E) = \frac{V_t - V_0}{V_0} \times 100

V_0 is the mean paw thickness before carrageenan injection. V_t is the mean paw thickness after carrageenan injection at time (t). The percentage oedema (%E) is the percentage difference between the paw thickness after and before carrageenan injection.

The per cent inhibition of the induced oedema in the treated group was calculated using the following formula:

Percentage Oedema Inhibition (%EI) = \frac{E_c - E_t}{E_c} \times 100

Where E_c is the oedema percentage of the control group and E_t is the oedema percentage in the treated group [13-14]. The protocol of this study complies with the ethical principles and guidelines for the care and the use of laboratory animals adopted by the "Research Ethics Committee", Faculty of Pharmacy, Mansoura University.

2.8. Statistical analysis:

The statistical significance of the all data obtained from the in vivo study (p<0.05) was computed with one-way ANOVA using Prism 5, GraphPad, Inc.

3. RESULTS AND DISCUSSION:

3.1. Calibration curve of Tolmetin sodium:

The absorbance values of Tolmetin sodium dilution series in PBS were measured at 323nm [Table 1]. The resulting straight line indicates that Tolmetin sodium obeys beer's law for the tested concentrations range [Fig.1].
Table 1: Calibration curve of Tolmetin sodium in Phosphate buffered saline λmax 323nm.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.249±0.008</td>
</tr>
<tr>
<td>6</td>
<td>0.356±0.019</td>
</tr>
<tr>
<td>8</td>
<td>0.464±0.012</td>
</tr>
<tr>
<td>10</td>
<td>0.580±0.032</td>
</tr>
<tr>
<td>12</td>
<td>0.700±0.029</td>
</tr>
</tbody>
</table>

3.2. In vitro release of the drug through dialysis membrane:

- The effect of polymer concentration on the release of the drug:

The cumulative per cent release of Tolmetin sodium from Eudragit E 100 containing patches after 3 hours was 89.6%, 77.8%, 68.9%, 60.3% from 4%, 6%, 8% and 10% (w/v) Eudragit E 100 containing patches respectively [Table 2]. Tolmetin sodium patches containing 4% (w/v) Eudragit E 100 gave the highest in-vitro release through the dialysis membrane followed by that containing 6% and 8% (w/v) and finally 10% (w/v) Eudragit E 100 containing patches [Fig. 2].
Similar release profiles of the drug were obtained with Eudragit L 100-55 transdermal patches using the same concentrations of the resin Eudragit E 100. The drug release after 3 hours was 102.4%, 98.7%, 96.0% and 87.5% from 4%, 6%, 8% and 10% (w/v) concentrations respectively [Table 2]. The descending order of the release of the drug from Eudragit L 100-55 patches was reversely related to Eudragit L 100-55 concentrations in the patches [Fig. 3].

On the other hand, Tolmetin sodium in HPMC containing patches of concentrations 4% and 6% in the preliminary study showed that there was no significant difference in the drug release profiles. So further study was made on concentrations 4%, 8% and 12%.

Tolmetin sodium patches containing 4% (w/v) HPMC gave the highest in-vitro release (98.6%) through the dialysis membrane followed by that containing 8% (90.4%) and finally 12% (w/v) HPMC containing patches (80.7%) [Table 2, Fig. 4].

The release of the drug decreased when the polymer concentration increased [Table 2]. This may be due to the adsorption of the polymer chains through the membrane surface forming a thin polymer layer, which obstructs drug release. Also, as the polymer concentration increases, its chains become more crowded resisting the diffusion of the drug through its network [15].

These results are in agreement with that reported by Sheth NS and Mistry RB (2011) who stated that the release of Propranolol decreased when the concentration of the polymers in the matrix increased [16]. Also, Patel BJ (2010) reported that the release of Carvidolol from the transdermal delivery system decreased with the increase of the polymer concentration [17].

![Figure 2: In-Vitro release of Tolmetin sodium from TDS using different percentages of Eudragit E 100 polymer.](image-url)
Figure 3: In-Vitro release of Tolmetin sodium from TDS using different percentages of Eudragit L 100-55 polymer.

Figure 4: In-Vitro release of Tolmetin sodium from TDS using different percentages of HPMC polymer.
Table 2: The release profile of Tolmetin sodium transdermal patches:

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Percentage Tolmetin sodium released from patches containing different polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eudragit E 100</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5</td>
<td>19.3</td>
</tr>
<tr>
<td>1.0</td>
<td>41.6</td>
</tr>
<tr>
<td>1.5</td>
<td>60.2</td>
</tr>
<tr>
<td>2.0</td>
<td>71.3</td>
</tr>
<tr>
<td>2.5</td>
<td>81.5</td>
</tr>
<tr>
<td>3.0</td>
<td>89.6</td>
</tr>
<tr>
<td>3.5</td>
<td>93.8</td>
</tr>
<tr>
<td>4.0</td>
<td>93.9</td>
</tr>
<tr>
<td>5.0</td>
<td>94.0</td>
</tr>
<tr>
<td>6.0</td>
<td>94.1</td>
</tr>
</tbody>
</table>

- The effect of polymer type on the release of the drug:

The cumulative per cent release of Tolmetin sodium patches containing different polymers was compared at polymer concentrations 4% and 8% after 3 hours. The release of Tolmetin sodium patches containing 4% of the polymers after 3 hours was 102.4%, 98.4% and 89.6% for Eudragit L 100-55, HPMC and Eudragit E 100 patches respectively [Table 2]. While Tolmetin sodium patches containing 8% of the polymers after 3 hours was 96.0%, 90.4% and 68.9% for Eudragit L 100-55, HPMC and Eudragit E 100 patches respectively [Table 2].

Figures 5 and 6 illustrate the effect of polymer type and the different release profiles of Tolmetin sodium patches from 4% and 8% Eudragit L 100-55, Eudragit E 100 and HPMC patches respectively. Eudragit L 100-55 patches gave the highest release profile followed by HPMC and finally Eudragit E 100.

The drug release from Eudragit L100-55 matrices occurs mainly by erosion, which increases with increasing in the pH of the dissolution media. This result is in agreement with that reported by
Durig T et al. 1999, Mehta KA et al. 2001 and Carelli V et al. 2000 [18-19-20]. The increased erosion may be due to the increase in ionization of methacrylic acid moiety present in the polymer creating electrostatic repulsion forces between Eudragit polymer chains and disrupting the matrix [21]. Eudragit L 100-55 is also readily soluble in neutral to weakly alkaline conditions and the enhanced solubility allows for the swelling of the polymer [22]. The dissolution media in the present study has pH 7.4 simulating the interstitial fluid, which is slightly alkaline promoting both swelling and erosion of the polymer matrix and thus increasing the drug release.

On the other hand, the drug release from Eudragit E 100 patches was the slowest compared with the other two polymers. This may be due to the electrostatic interaction between the anionic Tolmetin with the cationic Eudragit E100. This electrostatic interaction caused retardation of the drug release giving a more sustained release profile.

These results are in agreement with those stated by Bhise KS et al. (2007) who, found that there was interaction between the anionic naproxen and the cationic chitosan, which would have been a potential cause of retarded drug release [23].

Also, Eudragit E 100 was found to significantly increase acidic drugs release in acidic medium and slightly decrease their release rate at higher pH [24]. The increase in the drug release could be due to the increase in the micro-environmental pH in the polymer layer.

![Figure 5: In-Vitro release of Tolmetin sodium from TDS using 4% different polymers.](image)
3.3. In vivo evaluation of Tolmetin sodium patches containing Eudragit L 100-55:

The results of carrageenan induced rat paw oedema test are shown in (Table 3). The table shows the oedema percentage during 24-hour study for both the control and treated rat groups and the oedema inhibition percentages of the medicated group.

After carrageenan injection, there was inflammation and increase in paw thickness in both groups. As shown in (Table 3) the mean per cent of oedema in the control group animals was 93.0 ± 12.7 while in the medicated group animals it was 59.2 ± 9.9 after three hours. The mean per cent increase in oedema was higher in the control group than in the medicated group [Table 3, Fig. 7].

Table 3: The percentage oedema of the control and medicated groups and the percentage oedema inhibition of the medicated group in carrageenan induced rat paw oedema experiment:

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>% Oedema (Mean ± SD)</th>
<th>% Oedema Inhibition in the medicated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Medicated</td>
</tr>
<tr>
<td>1</td>
<td>31.3 ± 0.8</td>
<td>17.9 ± 13.3</td>
</tr>
<tr>
<td>2</td>
<td>72.4 ± 9.2</td>
<td>31.1 ± 9.2</td>
</tr>
<tr>
<td>3</td>
<td>93.0 ± 12.7</td>
<td>59.2 ± 9.9</td>
</tr>
<tr>
<td>4</td>
<td>92.4 ± 12.1</td>
<td>64.3 ± 12.0</td>
</tr>
<tr>
<td>5</td>
<td>86.3 ± 10.2</td>
<td>64.9 ± 14.9</td>
</tr>
</tbody>
</table>
Figure 7: Comparison between the percentage oedema of the control and medicated groups in carrageenan induced rat paw oedema experiment.

The swelling and increase in paw thickness was significantly lower ($p < 0.0001$) in the medicated group indicating the efficacy of the tested patches in inhibiting the carrageenan induced oedema.

Throughout the study the per cent increase in paw thickness in the medicated group remained lower than that of the control group indicating the sustaining effect of the drug against the carrageenan induced inflammation.

The obtained results are in agreement with that reported by Buritova J and Besson JM (1997) who observed that the relatively low dose of 0.3 mg/kg of lornoxicam produced a marked reduction of the carrageenan induced rat paw oedema. Also, Panchaxari DM et al. (2013) stated that Diclofenac diethylamine patches were effective in inhibiting carrageenan-induced inflammation as they showed immediate action without any lag time. In another study the anti-inflammatory effect of ethyl acetate fraction of root bark of *Cassia sieberiana* and indomethacin were tested using carrageenan induced rat paw oedema where a significant dose-dependent reduction in the percentage oedema formation was obtained compared with the controls. Sakat SS et al. (2014) confirmed the anti-inflammatory effect of diclofenac on carrageenan-induced oedema in rat paw. These authors reported that Diclofenac showed a
maximum anti-inflammatory activity at 2 and 3 h for the low (5 mg/kg) and high doses (20 mg/kg), respectively, and maintained the activity until the last time point analysed.

4. CONCLUSION

From the in vitro release data, it was found that the drug release increased when the concentration of the polymer decreased and vice versa.

The drug release results from Eudragit L 100-55 patches were higher than that from HPMC and Eudragit E 100 patches.

The induced oedema percentage in the control group after three hours was 93.0 %. Whereas the medicated group showed a significant decrease in the swelling of the paw (59.2 %) compared with the control.

The Tolmetin sodium transdermal patches under investigation in this study produced a significant anti-inflammatory effect.

5. REFERENCES: