INVESTIGATIONS ON ANTI-INFLAMMATORY ACTIVITY OF SOLID DISPERSION OF INDOMETHACIN PREPARED WITH SKIMMED MILK

INDOMETASININ AZ YAĞLI SÜT İLE HAZIRLANAN KATI DISPERSİYONUNUN ANTIENFLAMATUVA ARİTİMİSİNİN İNCELENMESİ

YALÇIN TOPALOĞLU¹, GÜLGÜN YENER² and GÜLSEL KAVALALI³

¹Department of Biopharmaceutic Pharmacokinetics
²Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Istanbul, 34452-Istanbul, Turkey
³Department of Herbal Medicine Research, Faculty of Cerrahpaşa Medicine, University of Istanbul, 34303-Istanbul, Turkey

Indomethacin (IND) is a nonsteroidal agent used for analgesic, anti-inflammatory and antipyretic effects in therapy. It is practically insoluble in water and has irritation on stomach. In order to modulate its irritation on stomach and increase solubility in water, physical mixture (PM) and solid dispersion (SD) of indomethacin with skimmed milk (SM) were prepared. Thus, in this study, by using "Hind Paw Oedema" method, anti-inflammatory activity of these formulations in comparison with IND were investigated.

Keywords: Indomethacin; Skimmed Milk; Physical Mixture; Solid Dispersion; Anti-inflammatory; Oedema

Anahtar kelimeler: Indometazin, Az Yağlı Süt; Fiziksel Karışım; Katı Dispersion; Anti-enflamatuvar; Ödem

Introduction

Indomethacin (IND) is a nonsteroidal anti-inflammatory, analgesic and antipyretic agent used in therapy. It is insoluble in water and creates disorders in gastro-intestinal system (1). It has been known that in order to enhance the solubility of nonsteroidal anti-inflammatory drugs, surface active agents or water soluble salts could be used (2). To improve dissolution and absorption rate of poorly soluble drugs, reduction of particle size have been reported (3). One of the ways to reduce particle size is forming solid dispersion (SD) (4).

In this study, in order to modulate IND's irritance on stomach and to enhance its solubility in water, physical mixture (PM) and SD of IND with skimmed milk (SM) were prepared and also "Hind Paw Oedema" method (5) was used to investigate the anti-inflammatory activity of these formulations by determining their oedema inhibition.

Materials and Methods

Materials

Indomethacin gift of Ratiopharm GmbH&Co., Germany, skimmed milk used as purchased, carrageenan (Sigma Type IV), all other reagents and chemical substances were of analytical grade.

Methods

In this study, IND, PM and SD of IND were investigated for their anti-inflammatory activity by using Hind Paw Oedema test.

1. Preparation of skimmed milk powder

After freeze-drying (SM was lyophilized until the sample's humidity reduced to maximum 3%. According to preliminary studies, lyophilization time was chosen as 72 h to reduce humidity), 25 ml SM yielded 2.615 g powder (SMP). The powder was sieved through 250 μm mesh.

2. Preparation of the physical mixtures

500 mg micronized drug was uniformly mixed with 2.615 g SMP using an agate mortar and pestle. The prepared PM were kept in a dessiccat over calcium chloride (0% relative humidity) at room temperature.

3. Preparation of the solid dispersions

500 mg IND was suspended in 25 ml SM. Suspension was mixed in a water bath having 50°C temperature for 30 minutes by using a magnetic stirrer. It was freezeed by keeping in fluid nitrogen bath and lyophilized (Leyvac GT 2-Leybold Heraeus). The yield of SD of IND with SM was sieved through 250 μm mesh.
Table 1. Anti-inflammatory activity of different formulations of IND (10 mg/kg) using carrageenan-induced paw oedema in rats

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>PD</td>
<td>N</td>
<td>PD</td>
<td>N</td>
<td>PD</td>
</tr>
<tr>
<td>Formulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (saline, i.p.)</td>
<td>5.1±0.18</td>
<td>6.08±0.25</td>
<td>0.98±0.5</td>
<td>6.73±0.46</td>
<td>1.23±0.24</td>
<td>7.52±0.57</td>
</tr>
<tr>
<td>IND (10 mg/kg, i.p.)</td>
<td>5.25±0.38</td>
<td>6.3±0.46</td>
<td>1.05±0.68</td>
<td>6.33±0.66</td>
<td>1.08±0.32</td>
<td>12.19</td>
</tr>
<tr>
<td>PM (10 mg/kg, i.p.)</td>
<td>5.1±0.46</td>
<td>6.12±0.28</td>
<td>1.02±0.7</td>
<td>5.72±0.18</td>
<td>0.62±0.22</td>
<td>49.59</td>
</tr>
<tr>
<td>SD (10 mg/kg, i.p.)</td>
<td>5.5±0.1</td>
<td>6.56±0.52</td>
<td>1.06±0.46</td>
<td>5.94±0.52</td>
<td>0.4±0.28</td>
<td>64.23</td>
</tr>
</tbody>
</table>

PD: Paw diameter (mm)
OI % : Oedema inhibition
* Significantly different from control value at p<0.05
**Significantly different from IND at p<0.05
4. Determination of anti-inflammatory activity of different formulations of IND using carrageenan-induced paw oedema in rats

Wistar albino male rats (Veterinary Faculty of Istanbul University) of 100-150 g weight were used in "Hind Paw Oedema" test. Rats were divided into four groups, one of being control. In order to produce inflammation, 0.1 ml 1% carrageenan solution in water were injected to hind paw's subplantar tissue of rats. Prior to this administration, paw diameters of rats were measured by dividers and recorded.

After carrageenan injections, IND (10 mg/kg) to group I, PM of IND with SMP (62.3 mg/kg PM equivalent to 10 mg/kg IND) to group II and SD of IND with SM (62.3 mg/kg SD equivalent to 10 mg/kg IND) to group III, both in physiological saline were injected intraperitoneally.

For control group, physiological saline was injected by the same way. After these injections, paw diameters were measured at 1, 2, 3, 20 and 24th hours. Percentages of oedema and oedema inhibition were calculated according to the formulas below.

\[ \text{Oedema\%} = \frac{N \times 100}{N} \quad \text{Oedema inhibition\%} = \frac{N - N_1 \times 100}{N} \]

N : After injection of carrageenan solution to control group, the measured paw diameters separately at (1, 2, 3, 20 and 24th hours) - paw diameters at the beginning.

N' : After injection of carrageenan solution to test groups, the measured paw diameters separately at (1, 2, 3, 20 and 24th hours) - paw diameters at the beginning.

Results and Discussion

It is obvious from Table 1 and Figure 1 that IND (10 mg/kg) significantly inhibited the carrageenan-induced paw oedema by 59.58% of the control value. Similarly SD and PM of IND with SM in a dose of 62.3 mg/kg (containing 10 mg/kg IND) showed a remarkable reduction in the paw diameters especially at the 3rd hour. The SD and PM of IND was found to cause percentage inhibition as 91.67 and 74.16 of the control values respectively.

It could be concluded that the anti-inflammatory activity of these formulations are significantly higher than that of plain IND. Thus means no lost of anti-inflammatory activity has been determined through preparation of SD of IND with SM, on the contrary a sustained and higher anti-inflammatory activity has been observed in tested period when IND was prepared as SD with SM.
References


Accepted: 28.03.1997