IN VIVO EVALUATION OF MISOPROSTOL FLOATING MICROSPHERES

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ABSTRACT

Prostaglandin (PG) has been reported to be an important anti-inflammatory and acid suppressive factor in the gastric mucosa. The objective of the study was to develop and evaluate a stomach specific drug delivery system for controlled release of Misoprostol a PG analogue for gastric ulcer induced NSAIDs. Floating microspheres were prepared by emulsification-solvent evaporation method using ethyl cellulose as a polymer, carboxyl as mucoadhesive polymer. Ulcers were induced by the oral administration of absolute ethanol (5 mL/kg) to 24 h fasted Wistar male rats (n=8), weighing 200 g. Sodium bicarbonate solution, misoprostol solution and drug loaded microspheres were tested. Formulations were administered orally 1h before the administration of ethanol. Prior to the oral administration, rats were anesthetized with ethyl ether. After 2 h of ethanol administration, animals were sacrificed; the stomachs were removed, opened along the greater curvature and examined for lesion measurements. Ulcer indexes (UI) were calculated. The in vivo evaluation showed that ulcer index values were 0.61 ± 0.14 for the sodium bicarbonate solution, 0.58 ± 0.18 for the misoprostol and 0.11 ± 0.06 for the misoprostol-loaded microspheres. The Kruskal-Wallis test detected statistical differences (p =0.002) between the ulcer indexes. The multiple analyses (Student-Newman-Keuls) showed that the misoprostol loaded microspheres presented a gastric ulcer index statistically lower than that of the sodium bicarbonate solution (p = 0.001) and the misoprostol solution (p = 0.021). The percentages of ulceration inhibition were 31 and 75% after the administration of misoprostol solution and microspheres, respectively. In conclusion, the in vivo evaluation showed that the microspheres presented ulcer index lower than the solutions, showing that misoprostol-loaded microspheres were efficient in protecting the stomach against ulcer formation.

Key words: Misoprostol, microspheres, ulcer index.

INTRODUCTION

Misoprostol is a drug used for the prevention of non steroidal anti inflammatory drug (NSAID) induced gastric ulcers. Pharmacologically, misoprostol is a synthetic prostaglandin E1 (PGE1) analogue. It is an effective anti-ulcer agent and also has oxytocic properties (Karim et al., 1987). This drug as methyl 7-[(1R,2R,3R)-3-hydroxy-2-[1(E)-4-hydroxy-4-methylocyclopentyl-1-en-1-yl]-5-oxocyclopentyl]heptanoate (Figure 1).

The naturally occurring prostaglandin E was discovered to inhibit the gastric acid secretion in 1967 by Robert et al. However naturally occurring prostaglandins have 3 drawbacks that have hindered clinical applications: 1. Rapid metabolism manifested as a lack of oral activity and short duration of action when given parenterally; 2. Incidence of numerous side effects; and 3. Clinical instability leading to short life (Collins et al., 1985).

Misoprostol is extensively absorbed and undergoes rapid de-esterification by the liver to form the free acid which is responsible for its clinical activity (Schoenhard et al., 1985). Misoprostol has both antisecretory (inhibiting the gastric acid secretion) and mucosal protective properties (Wilson et al., 1986). NSAIDS inhibit prostaglandin synthesis and a deficiency of prostaglandins within the gastric mucosa may diminish bicarbonate and mucus secretion and contribute to the mucosal
damage caused by these agents (Zeman et al., 1997). Because of its potent anti-secretory and cytoprotective effects on the gastric and duodenal mucosa, misoprostol is an effective drug in the treatment of gastric and duodenal ulcers (Tang et al., 2002a). In addition, misoprostol has been shown to protect the gastric duodenal mucosa from the damaging effects of alcohol and non-steroidal anti-inflammatory drugs (Watkinson et al., 1987). The aim of this work was to test the in vivo capacity of misoprostol microspheres to protect the gastric mucosa against ulcer formation.

**METHODOLOGY**

Misoprostol was gifted from Siris pharmaceuticals, Hyderabad. HPMC K 100M and Ethyl cellulose were gifted from Matrix labs, Hyderabad. All the other chemicals and reagents used were of analytical grade.

**Preparation of floating microspheres**

Microspheres containing Misoprostol were prepared by a non-aqueous solvent evaporation method. Drug, HPMC and EC were mixed in the mixture of dichloromethane and ethanol at a 1:1 ratio (Rajeev et al., 2010). The slurry was slowly introduced into 100 mL of liquid paraffin containing 1% Tween 80, maintained at a temperature of 30-40°C and subsequently stirred at ranging agitation speed for 45 min and the solvent evaporated completely, and filtered by using filter paper. The microspheres obtained were washed repeatedly with petroleum ether until free from oil. The collected microspheres were dried at room temperature and subsequently stored in desiccators.

**In vivo anti-ulcer activity**

**In vivo protocol**

All the groups of animal were kept for over night fasting, fed only with the tap Water. Ulcers were induced by the oral administration of absolute ethanol (5 mL.kg⁻¹) to 24 h fasted Wistar male rats (n = 8), weighing 200 g. The groups are described in table 1. Formulations (14 μg.kg⁻¹ of drug) were administered orally 1 h before the administration of ethanol. Prior to the oral administration, rats were anesthetized with ethyl alcohol ether. After 2 h of ethanol administration, animals were sacrificed the stomachs were removed, opened along the greater curvature and examined for lesion measurements (Raffin et al., 2000). The observation was made for any bulging or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposing for evaluation. The ulcer scores (US) were calculated as the arithmetic mean for each treatment.

**Calculation of ulcer score – ulcer index**

The stomach was opened along the greater curvature and washed slowly under running tap water. It was put on a glass slide and observed under 10x magnification for ulcers. The ulcers were scored as shown in table II. Mean ulcer score in each group was calculated and was designated as ulcer index and percentage was calculated as:

\[
% \text{ Protection} = \frac{(C-T/C) \times 100}{C}
\]

Where  
\[C = \text{ulcer index in control group}\]
\[T = \text{ulcer index in treated group}\]

**RESULTS AND DISCUSSION**

**In vivo anti-ulcer activity**

The in vivo evaluation showed that ulcer index were 0.61 ± 0.14 for the sodium bicarbonate solution, 0.58±0.18 for the misoprostol and 0.11 ± 0.06 for the misoprostol-loaded microspheres (Figure 2). The Kruskal-Wallis test detected statistical differences (p=0.002) between the ulcer indexes. The multiple analyses (Student-Newman-Keuls) showed that the misoprostol loaded micro particles presented a gastric ulcer index statistically lower than the sodium bicarbonate solution (p=0.001) and the misoprostol solution (p=0.021). Oral administration of ethanol to the control groups clearly showed hemorrhagic lesions developed in the glandular portion of the stomach (Figure 3). The percentages of ulceration inhibition were 31 and 75% after the administration of miso-prostol solution and microspheres respectively.

**Statistical analysis**

One-way analysis of variance was employed for the comparison of the experimental data. The non-parametric test Kruskal-Wallis was used for the in vivo data.
Table I. Groups of Rats (Control 1, Control 2 and Treatment) for the In Vivo Anti-Ulcer Activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Administered Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>Sodium bicarbonate solution (4%)</td>
</tr>
<tr>
<td>Control 2</td>
<td>Misoprostol dissolved in water</td>
</tr>
<tr>
<td>Treatment</td>
<td>Microspheres dispersed in water</td>
</tr>
</tbody>
</table>

Table II. Ulcer scoring

<table>
<thead>
<tr>
<th>Observations on stomach</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal colored stomach</td>
<td>0.0</td>
</tr>
<tr>
<td>Red coloration</td>
<td>0.5</td>
</tr>
<tr>
<td>Spot ulcers</td>
<td>1.0</td>
</tr>
<tr>
<td>Hemorrhagic streaks</td>
<td>1.5</td>
</tr>
<tr>
<td>Ulcers (≥ 3 ≤ 5 mm)</td>
<td>2.0</td>
</tr>
<tr>
<td>Ulcers (&gt; 5 mm)</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Figure 1. Chemical structure of misoprostol

Figure 2. Ulcer indexes for the sodium bicarbonate solution (SB), Misoprostol solution (MS) and misoprostol microspheres (MM).
Multisample comparison was performed using Student-Neuman-Keuls test. (Raffin et al., 2006)

CONCLUSION
The \textit{in vivo} anti-ulcer evaluation demonstrated that microspheres were able to reduce ulcer formation caused by oral administration of ethanol (Figure 3). Ethanol-induced gastric lesions are due to stasis in gastric mucosa, which contributes to the development of the hemorrhage and necrotic aspects of the tissue injury. The gastric lesions caused by ethanol have been attributed to free radical formation and subsequent formation of lipid peroxidation products. The induction of ulcers by ethanol was considered a good model to evaluate the effect of Misoprostol. The \textit{in vivo} evaluation showing that Misoprostol microspheres were efficient in protecting the stomach against ulcer formation.

ETHICAL APPROVAL OF STUDIES
The protocol of the \textit{in vivo} experiments was approved by the Ethical Committee (IAEC, 1358/ac/10/CPCSEA) Vishnu Institute of Pharmaceutical Education and Research, Medak, Andhra Pradesh, India.

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