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RESEARCH ARTICLE

Comparative pharmaceutical study on colon targeted micro-particles of celecoxib: in-vitro–in-vivo evaluation

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Abstract

In order to target celecoxib which is a COX2 inhibitor, with potentials in the prevention and treatment of colitis and colon cancer, it was formulated as microparticles using the solvent/evaporation method and various pH-dependent Eudragit polymers. The in-vitro evaluation of the prepared microparticles showed spherical and smooth morphology. The encapsulation efficiency and yield were high, indicating that the method used is simple and efficient at this scale. The in-vitro release study showed no release in the acidic medium for 2 h followed by the release of the drug in pH 6.8 in case of Eudragit L100-55 and L100 and pH 7.4 in case of Eudragit S100. The pharmacokinetic parameters were calculated and method validation was performed to insure that it is suitable and reliable. Pharmacokinetic parameters were investigated by determining the C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0–t</sub>, K<sub>el</sub>, and t<sub>1/2</sub> of the drug as a suspension and as microparticles. There was a significant difference (p<0.05) in T<sub>max</sub> between the drug as a suspension and as microparticles. The effect of celecoxib on the degree of inflammation was examined on acetic acid induced colitis rat model and the drug was given as a suspension and as microparticles. The evaluation was done using macroscopical, microscopical and biochemical examination. There was a significant difference between the acetic acid control group and the treatment groups regarding all examination criteria in the order microparticles formulated using Eudragit S100 followed by Eudragit L100-55 while microparticles using Eudragit L100 and drug suspension showed almost the same results.

Introduction

Many pathological conditions affect the colon and they range in severity from simple constipation or diarrhea to the more serious inflammatory bowel diseases such as ulcerative colitis or Crohn’s disease moving up to colon cancer (El-Kamel et al., 2008). Many orally administered drugs are not effective or absorbed before reaching the colon. According to this, oral dosage forms that deliver the drug to the colon rather than the upper GIT are very beneficial in the treatment of colon diseases by delivering the drug to the site of action (Jackson et al., 2015). This ensures high local concentration while minimizing the side effects arising from the release of the drug in the upper GIT or unnecessary systemic absorption (Crcaerveška et al., 2008; Tian et al., 2016).

Non-steroidal anti-inflammatory drugs (NSAIDs) are of the commonly used agents in the control of the acute attacks of inflammatory bowel diseases (IBD) and to prevent further attacks during remission (Makhlof et al., 2009). They also showed potentials in the prevention and treatment of colitis and colon cancer (Myers et al., 2001; Yamazaki et al., 2002; Jung et al., 2005). But, the oral administration of these drugs lead to high absorption from the upper GIT and causes systemic side effects. According to this, it is preferable to deliver the drug to the site of action directly.

NSAIDs mechanism of action depends on blocking the production of prostaglandins by inhibiting the cyclooxygenase enzyme (Smith & Willis, 1971; Paulson et al., 2001). There are two cyclooxygenase enzymes, namely COX-1 and COX-2 (Smith & Willis, 1971; Masferrer et al., 1994). COX-2 is induced and highly expressed in association with inflammation (Raz et al., 1988; Masferrer et al., 1994) and it was reported that the selective inhibition of COX-2 will have the anti-inflammatory effect without the GIT side effects of traditional NSAIDs (Simon et al., 1999; Silverstein et al., 2000).

Celecoxib is a selective cyclooxygenase (COX-2) inhibitor. It is highly lipophilic and poorly soluble. It has large volume of distribution and this may reflect low bioavailability (Ghorab et al., 2011; Song et al., 2014). So, by targeting celecoxib to the colon, this will lead to high localization of the drug at the site of action and enhance the therapeutic efficiency.
There are many techniques for drug targeting. The micro-particulate system where the particle size ranges from one micron to few millimeters using pH-dependent polymers represents one of the simplest approaches (Dang et al., 2015).

The aim of this work is to formulate celecoxib as colon-targeted microparticles using different pH-dependent polymers employing a simple and economic method. In-vitro evaluation of the prepared microparticles followed by estimation of the pharmacokinetics of celecoxib after a single oral dose as a suspension and as colon-targeted microparticles using different pH sensitive polymers. Finally, the in-vivo therapeutic efficacy of celecoxib as a suspension and as colon-targeted microparticles is to be evaluated and compared in acetic acid-induced colitis rat model. The evaluation is done using macroscopic, microscopic and biochemical tests where the TNF-α and prostaglandin E2 levels are estimated in addition to the determination of iNOS and MPO activities.

Materials and methods

Materials

Celecoxib was a gift sample from Memphis pharmaceutical company (Cairo, Egypt). Eudragit S100, Eudragit L100-55, and Eudragit L100 were a generous gift from Evonik (Darmstadt, Germany). Sorbitan monooleate (span 80), Tween 20, n-hexane, liquid paraffin, absolute ethanol, methanol and sodium monohydrogen phosphate and sodium dihydrogen phosphate were purchased from Sigma Aldrich (St. Louis, MO). All other chemicals used were of analytical grade.

Animals

A total of 60 male wistar rats (200–300 g) were used in this study. The rats were housed individually in a rack mounted with wire mesh cages to prevent coprophagia. All rats were exposed to the same environmental conditions and maintained on proper diet and water. All procedures were approved by research ethics committee for experimental and clinical studies (Serial number PI 233), Faculty of Pharmacy, Cairo University.

Preparation of celecoxib microparticles

Three grams of each polymer were dissolved in 30 mL absolute ethanol, followed by dissolving of the drug. The alcoholic solution was added to 200 mL liquid paraffin containing 1% w/w of span 80 while stirring with a mechanical stirrer at 700–900 rpm at room temperature. Stirring was conducted for 12 h or until all the solvent evaporates. The prepared microparticles were separated by filtration, washed three times with n-hexane to harden the micro-particles, then left to dry at room temperature for at least 24 hours (Kendall et al., 2009).

In-vitro characterization of the prepared microparticles

Particle morphology

The shape and surface morphology of the prepared celecoxib microparticles were examined by scanning electron microscopy. Samples of the microparticles were sputter coated with gold and photographed using a scanning electron microscope (Quanta FEG 250; FEI, Hillsboro, OR).

Particle yield and particle size

The yield of the microparticle production was calculated using the following formula

\[
Yield = \frac{\text{Observed mass of microparticles produced}}{\text{Theoretical mass of microparticles expected}} \times 100
\]

The mean diameter of the prepared microparticles was determined using light microscopy.

Drug loading and encapsulation efficiency

Drug-loaded microparticles (30 mg) were dissolved in 50 mL methanol with the aid of brief sonication. The resulting methanolic solution was made to 250 mL using phosphate buffer (pH 6.8) containing 1% v/v tween 20. The resulting solution was analyzed for celecoxib spectrophotometrically at 257 nm. Drug-loading and encapsulation efficiency were calculated using the following equations: (drug mass per gram of polymer) and expressed as (observed drug loading/theoretical drug loading × 100) respectively.

In-vitro drug release

In-vitro dissolution was carried at 37°C and 900 mL dissolution medium using USP II (paddle) apparatus at 100 rpm. All samples were tested for 2 h in 0.1N HCl (pH 1.2), then at pH 6.8 for Eudragit L100-55 and Eudragit L 100 microparticles and pH 7.4 for Eudragit S100 microparticles. Aliquots each of 10 mL from the dissolution medium were withdrawn at 0.5, 1, 2, 3, 4, 6 and 8 hours’ time intervals and replaced by 10 mL of dissolution medium. The amount of celecoxib released was measured spectrophotometrically at 255 nm.

Dissolution stability study of the prepared microparticles

Stability of the selected microparticles was measured by evaluating the changes in dissolution profile. Celecoxib microparticles were stored in clear glass vials at room temperature, 40°C and 60°C for a period of 12 weeks. Samples were withdrawn and the drug release profiles were assessed after 30, 60 and 90 days.

In vivo evaluation of celecoxib microparticles

Pharmacokinetic analysis

The study was carried out to compare the pharmacokinetic parameters of the selected celecoxib-loaded Eudragit microparticles to the oral suspension of the drug, following the administration of a single dose of 5 mg/kg of each. For this study four groups (3 animals/group) were fasted over night with free access to water. Each group received a single dose of celecoxib (5 mg/kg) by gastric intubation, where group A received free celecoxib dispersion, while groups B, C and D received the selected celecoxib-loaded Eudragit microparticles formulae. All treatments doses were dispersed in 1% CMC solution. Serial blood samples were collected directly through retro-orbital puncture from each rat into heparinized
tubes at 5, 15, 30 min, 1, 2, 4, 6, 8 and 24 h post dose. The plasma celecoxib concentration was measured by a validated LC-MS/MS method. Pharmacokinetic parameters were investigated by determining the $C_{\text{max}}$, $T_{\text{max}}$, AUC$_{0-t}$, $K_{\text{el}}$, and $t_{1/2}$ using Winnonlin software (Certara USA, Inc., Princeton, NJ).

**Liquid chromatography**

Liquid chromatograph, autosampler, and degasser (Agilent 1200 series; Agilent Technologies, Santa Clara, CA) were used. These components were directly controlled by the Mass Hunter data system. The mobile phase consisted of ammonium acetate pH 5: Methanol (20:80 v/v) and was pumped at a flow rate of 0.55 ml/min. The column was Phenomenex Luna C8, 5 μm, 50 mm x 4.6 mm and was operated at ambient temperature.

**Mass spectroscopy**

The LC analyses were performed using Mass spectrometer (Agilent 6410 Triple Quad; Agilent Technologies, Santa Clara, CA). The ion source was with the following parameters; gas temperature 350°C, gas flow rate 10 l/min, nebulizer pressure 40 PSI and capillary voltage 5000V. The scanning mode was in positive ionization mode (ESI) at 380.1 and 316.9 for celecoxib and fenofibric acid, respectively, and the analytical data were acquired by Mass Hunter software.

**Induction of experimental colitis in rats**

For the *in-vivo* study the animals were fasted for 24 hr, with access to water before the induction of colitis. The induction of colitis was performed by sedating each rat using ether. Two milliliters of acetic acid (3% v/v in 0.9% saline) was infused slowly using a polyethylene tube (2 mm in diameter), which was inserted rectally into the colon to a distance of 8 cm. The acetic acid was retained in the colon for 60s after which the fluid was withdrawn (Millar et al., 1996; El-Medany et al., 2005). The animals were randomly divided into 6 groups each consisting of eight rats, normal control group, acetic acid control group and 4 drug-treated groups where they received celecoxib at a dose of 5 mg/kg as free powder or microparticles prepared by different pH-dependent polymers. The drug and microparticles were all suspended in 1% CMC and administered orally in a volume of 0.5 mL/100 g body-weight. The dose was given twice daily starting 24 h after the induction of colitis and continued for five consecutive days. After the completion, the rats were killed and colonic biopsies were taken for macroscopic scoring, histopathological examination and biochemical studies.

**Table 1. Celecoxib microparticles using different pH dependent polymers.**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Milligrams of celecoxib</th>
<th>Ratio of drug: polymer</th>
<th>Polymer used</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100</td>
<td>1:30</td>
<td>Eudragit S100</td>
</tr>
<tr>
<td>F2</td>
<td>300</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>600</td>
<td>1:5</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>100</td>
<td>1:30</td>
<td>Eudragit L100-55</td>
</tr>
<tr>
<td>F5</td>
<td>300</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>600</td>
<td>1:5</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>100</td>
<td>1:30</td>
<td>Eudragit L100</td>
</tr>
<tr>
<td>F8</td>
<td>300</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>600</td>
<td>1:5</td>
<td></td>
</tr>
</tbody>
</table>

All microparticles were prepared using 1% w/w Span 80 as emulsifier.
Assessment of colitis

Body weight

The weight of each rat was recorded to assess the effect of acetic acid-induced colitis on body weight before and after treatment with celecoxib (5 mg/kg) as suspension and as micro-particles prepared using the different pH-dependent polymers.

Macroscopic scoring

Six centimeters of colon extending proximally for 2 cm above the anal orifice was removed, then washed with normal saline, split longitudinally and pinned on a plate. An independent observer scored the macroscopic appearance of the colonic mucosa. An arbitrary scale ranging from 0 to 4 was used. 0 was given for no macroscopic change, 1 for mucosal erythema only, 2 for mild mucosal edema, slight bleeding or small erosions, 3 for moderate edema, bleeding ulcers or erosions, and finally 4 for severe ulceration, erosions, edema and tissue necrosis (Millar et al., 1996).

Histopathological study

Full thickness biopsy specimens were fixed in 10% formal saline for 24 h. Washing was done in tap water then serial dilutions of alcohols (methanol, ethanol and absolute ethanol) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for examination by light microscopy (Bancroft & Dawsom, 1996).

Biochemical study

Colonic samples were stored immediately at −20 °C until analysis. The myeloperoxidase (MPO) and total nitric acid synthetase (iNOS) activities were measured. Also, tumor necrosis factor alpha (TNF-α) and prostaglandin E2 levels were estimated.

Measurement of tumor necrosis factor alpha (TNF-α) production

The level TNF-α was measured using RayBio® Rat TNF-alpha ELSA (Enzyme-Linked Immunosorbent Assay kit) (Norcross, GA).

Measurement of prostaglandin E2 production

The level of Prostaglandin E2 (PGE2) was estimated using Rat Prostaglandin E2 (PGE2) ELISA Kit (Cusabio, Baltimore, MD) this immunoassay kit allows for the in vitro quantitative determination of rat PGE2 concentrations in tissue homogenates.

Determination of myeloperoxidase (MPO) activity

The MPO activity was determined using the HK105 rat MPO ELISA kit (Hycult Biotech, Uden, the Netherlands).
Determination of total nitric oxide synthetase enzyme activity

Rat inducible nitric oxide synthase, iNOS ELISA Kit (Eiaab, China) was used to determine the enzyme activity.

Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M). Statistical group analysis was performed with graph pad prism version 5 (Graphpad Software Inc., San Diego, CA). One–way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups. \( p < 0.05 \) was considered significant.

Results and discussion

Spherical, uniform, smooth surfaced microparticles were successfully produced using emulsification/solvent evaporation process. The different formulae of celecoxib microparticles are listed in Table 1. Scanning electron micrographs of celecoxib-loaded Eudragit S100, Eudragit L100-55 and Eudragit L100 microparticles are shown in Figure 1.

The microparticles yield from the three polymers was above 80% with no significance difference between the three polymers (\( p < 0.05 \)). The microparticles using the three Eudragit polymers were capable of encapsulating celecoxib at high efficiency. The highest encapsulation efficiency was for Eudragit S100, followed by the other two polymers with no real difference between the three polymers. This was different from the findings of Kendall et al. (2009), where the microparticles prepared from Eudragit L100-55 showed the highest encapsulation efficiency followed by the other two polymers. This could be attributed to that the microparticles prepared in this study using the polymer Eudragit S100 were of larger particle size and as the particle size increases, the smaller the total interface area and less likely the drug is to migrate to liquid paraffin phase leading to higher encapsulation efficiency. The properties of the prepared microparticles are given in Table 2.

The method used was simple and applicable to a number of pH-responsive polymers. It was adapted from the method used by Kendall et al. (2009) to prepare microparticles by emulsification/solvent evaporation, but the emulsifier arlacel 83 was substituted by span 80 after various trials. Span 80 showed better emulsion stability and better quality of the produced microparticles. The high-encapsulation efficiency and microparticle yield suggested that the process is efficient and economical at this scale.

The microparticles prepared using the three polymers did not release any drug at acidic medium pH 1.2 for two hours. The microparticles prepared using Eudragit S100 released celecoxib within 15 minutes after the pH changed to 7.4. While the microparticles prepared using the Eudragit L polymers released celecoxib within 30 minutes after the pH change to 6.8. This was in accordance with the results of Chourasia & Jain (2004), and is demonstrated in Figure 2.

Both Eudragit L microparticles showed no drug release in the acidic medium, and this was the opposite from the findings of Eerikäinen et al. (2004); Devarajan & Sonavane (2007); Paola et al. (2008), where the Eudragit L

Figure 2. In-vitro release of celecoxib from the prepared microparticles.
microparticles released more than 10% of the drug in acidic medium. Also, the finding of Kendall et al. (2009) stated that the L 55 released less than 10%, while the microparticles in this study showed no release of the drug in 2 h exposure to acidic medium and this totally conformed to the specification of the United States Pharmacopeia 24 for enteric-coated products at all drug-to-polymer ratios.

The release profile of celecoxib from the selected microparticles prepared according to formulae F3, F6 and F9 after storage for 12 weeks at ambient and accelerated conditions was not changed. These results indicated that these microparticles are stable at these storage conditions. The glass transition temperature of the three polymers (T_g), which is above 130 ± 5°C for Eudragit S100 and Eudragit L100 and 96 ± 5°C for Eudragit L100-55, is directly linked to this high stability. This high stability offers an industrial advantage over some other solid dispersions that commonly suffer instability upon storage (Serajuddin, 1999). These results also conform to the findings of Kendall et al. (2009). The results are illustrated in Figures 3–5.

The average plasma celecoxib concentration–time curves after a single oral dose of 5 mg/kg as a suspension and as Eudragit microparticles according to F3, F6 and F9 are shown in Figure 6.

The pharmacokinetic parameters of celecoxib were calculated from individual curves and mean values are given in Table 3.

The statistical analysis of C_max, AUC0–∞, K_d and t_1/2 showed no significant difference (p<0.05), but there was a slight increase in the C_max of celecoxib-loaded Eudragit microparticles prepared according to F3 and F6. The mean T_max value for the drug suspension was 2.6 h and for the drug prepared as microparticles according to F3, F6 and F9 were

![Figure 3. In-vitro release of celecoxib from Eudragit S100 micro-particles at different storage temperatures (results are average of 3 measurements).](image)

![Figure 4. In-vitro release of celecoxib from Eudragit L100-55 micro-particles at different storage temperatures (results are average of 3 measurements).](image)
6.67, 5.5 and 4h, respectively. There was a significant difference between them at \( p < 0.05 \). By comparing the individual AUC_{0-\infty} values of the drug prepared as microparticles according to F3, F6 and F9 with the drug suspension to determine the relative bioavailability, there was an increase in the bioavailability of celecoxib prepared as Eudragit microparticles.

The results obtained from the pharmacokinetic analysis suggested that the delay in \( T_{\text{max}} \) indicated delayed release and slow release of the drug from the microparticles, leading to the localization of the drug at the site of action which is the colon and may affect the extent of drug absorption. Also, the increase in the bioavailability indicated more than 60% increase in the drug oral bioavailability by the microparticle formulae using Eudragit S100 and Eudragit L100-55.

Regarding the assessment of colitis, the macroscopic examination of the untreated group showed severe damage with ulceration ranging from moderate to severe. Also, bleeding erosions were observed and some specimen showed tissue necrosis, this was accompanied with bleeding diarrhea and weight loss.

Regarding the treated groups using formulae (F3), (F6), (F9) and celecoxib suspension, the least damage was observed in the group treated with F3 followed by F6 then F9 and the drug suspension. This was in accordance with the weight loss of the four groups. The least weight loss was observed in the group treated with formula F3 followed by F6, and finally F9 and the drug suspension as shown in Figures 7 and 8.

The histopathological findings of the control group showed no histopathological alteration and normal histological structure of the mucosa, submucosa and muscularis. The acetic acid-induced colitis group revealed destructed mucosa with focal inflammatory cells associated with lymphoid hyperplasia in the follicle at the submucosa. Huge improvement was observed in the group treated with F3 where the mucosal layer was histologically intact while the submucosa showed few lymphoid follicle hyperplasias. Moderate improvement was observed in the group treated with F6 while mild improvement was observed in the group treated with F9 and the drug suspension as demonstrated in Figure 9.

TNF-\( \alpha \) (tumor necrosis factor-\( \alpha \)) is an inflammatory marker secreted by macrophages, monocytes, neutrophils, T-cells, NK-cells following their stimulation by bacterial lipopolysaccharides (Gomes et al., 2016). By performing pairwise comparison among the six tested groups, The TNF-\( \alpha \) level showed significant difference among the six tested groups \((p < 0.05)\). It was clear that the mean value was significantly higher in the acetic acid control group as compared to normal control group. There was a significant decrease in its level in the treated groups. The highest decrease was observed in the group treated with F3 followed by the group treated with F6 and the least decrease was observed in the two groups treated with F9 and the drug suspension.

Prostaglandin E\(_2\) is thought to be the most dominant pro-inflammatory prostaglandin and plays a major role in inflammatory symptoms (Sugita et al., 2016). It was clear that the level of prostaglandin E\(_2\) were significantly elevated in acetic acid control group when compared to normal control group \((p < 0.05)\). However, there was a significant decrease in its levels in the treated groups in the order F3>F6>F9 and drug suspension.

The activity of MPO, which is found in neutrophils, can be used for evaluating the degree of inflammation in the intestine (Tozaki et al., 2002). The level of MPO activity in acetic acid control group showed a significant increase when compared to normal control group \((p < 0.05)\), while the level of MPO activity showed a significant decrease in the same pattern for the treated groups.

Induced NO synthesis was reported in inflammatory responses initiated by microbial products or autoimmune reactions also to be the major NO synthase involved in NO production during inflammatory conditions (Yuan et al., 2016). It was clear that the level of iNOS activity was significantly elevated in the acetic acid control group as
Table 3. Pharmacokinetic parameters after oral administration of celecoxib microparticles.

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Drug suspension</th>
<th>F3</th>
<th>F6</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{max}}) (ng/ml)</td>
<td>293.3 ± 14.99</td>
<td>324.7 ± 5.21</td>
<td>316.3 ± 13.38</td>
<td>294.0 ± 7.10</td>
</tr>
<tr>
<td>(\text{AUC}_{0-\infty}) (ng.h/ml)</td>
<td>3793.97 ± 623.3</td>
<td>5699 ± 413.20</td>
<td>5867.58 ± 477.80</td>
<td>4641.74 ± 669.80</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>2.67 ± 0.67</td>
<td>6.33 ± 0.33*</td>
<td>5.66 ± 0.35*</td>
<td>4.00 ± 1.00*</td>
</tr>
<tr>
<td>(K_{\text{el}}) (h(^{-1}))</td>
<td>0.078 ± 0.03</td>
<td>0.071 ± 0.01</td>
<td>0.073 ± 0.01</td>
<td>0.069 ± 0.01</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>10.64 ± 2.59</td>
<td>9.85 ± 0.28</td>
<td>9.67 ± 0.88</td>
<td>10.41 ± 0.88</td>
</tr>
<tr>
<td>Relative bioavailability (%)</td>
<td>——</td>
<td>162.71 ± 40.20</td>
<td>163.7 ± 30.27</td>
<td>125.8 ± 16.40</td>
</tr>
</tbody>
</table>

*All results are the mean ± SEM of 3 values.

Figure 6. (A) Plasma concentration time curve of celecoxib oral suspension (5 mg/kg) vs celecoxib-loaded eudragit S100 microparticles, (B) celecoxib loaded Eudragit L100-55 microparticles, (C) celecoxib-loaded Eudragit L100 microparticles, (D) plasma concentration time curve of celecoxib-loaded Eudragit polymers F3, F6 and F9 and (E) plasma concentration time curve of celecoxib as suspension and as Eudragit microparticles.
Figure 7. Macroscopic scoring of rat rectal biopsy of normal — acetic acid-induced colitis and after treatment with celecoxib (5 mg/kg) as suspension and as Eudragit-loaded microparticles.

Table 4. The effect of celecoxib formulae on the biochemical tests results.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dose (mg/Kg p.o)</th>
<th>TNF-α level (pg/g tissue)</th>
<th>PGE2 levels (ng/g tissue)</th>
<th>NOS activity (ng/g tissue)</th>
<th>MPO level (units/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>6</td>
<td>—</td>
<td>5.48 ± 0.52</td>
<td>4.383 ± 0.50</td>
<td>4.48 ± 0.49</td>
<td>1.78 ± 0.17</td>
</tr>
<tr>
<td>Acetic acid control</td>
<td>6</td>
<td>—</td>
<td>39.10 ± 3.75</td>
<td>39.18 ± 2.53</td>
<td>40.75 ± 3.25</td>
<td>12.60 ± 0.78</td>
</tr>
<tr>
<td>Celecoxib loaded Eudragit S100 microparticles (Gp.1)</td>
<td>6</td>
<td>5</td>
<td>15.58 ± 0.62</td>
<td>14.55 ± 0.57</td>
<td>17.50 ± 0.60</td>
<td>5.46 ± 0.28</td>
</tr>
<tr>
<td>Celecoxib loaded Eudragit L100-55 microparticles (Gp.2)</td>
<td>6</td>
<td>5</td>
<td>21.00 ± 0.87</td>
<td>20.02 ± 0.77</td>
<td>22.72 ± 0.77</td>
<td>7.58 ± 0.21</td>
</tr>
<tr>
<td>Celecoxib loaded Eudragit L100 microparticles (Gp.3)</td>
<td>6</td>
<td>5</td>
<td>28.97 ± 0.92</td>
<td>28.48 ± 0.84</td>
<td>30.58 ± 0.81</td>
<td>9.43 ± 0.35</td>
</tr>
<tr>
<td>Celecoxib as suspension (Gp.4)</td>
<td>6</td>
<td>5</td>
<td>30.73 ± 2.67</td>
<td>30.08 ± 1.12</td>
<td>32.32 ± 1.04</td>
<td>10.27 ± 0.48</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M of 6 observations. The vehicle or drug was administered orally twice daily for 5 days, 24 h after the induction of colitis.
compared to the normal control group. A significant marked decrease was observed in the group treated with F3 followed by the group treated by F9, and finally the groups treated with F9 and the drug suspension, as shown in Tables 4 and 5.

Based on the biochemical test results and histopathological findings, the prepared microparticles of the drug (F3, F6) reduced the inflammation and damage to the colon. This reduction was more compared to the drug suspension and F9. Celecoxib reduced the severity of colonic injury induced by acetic acid (Cuzzocrea et al., 2001; El-Medany et al., 2005) and the prepared microparticles increased and localized the drug effect on the colon.

In conclusion, the results suggest that pH-sensitive polymers delayed the release of celecoxib from the prepared microparticles, and that localized the drug in the colon and was accompanied by higher reduction of colon inflammation induced by acetic acid, which was clear with Eudragit S100 and Eudragit L100-55 microparticles and to lesser extent Eudragit L100-55.

Based on this study, targeting celecoxib to the lower GIT and colon using microparticles prepared using pH-dependent polymers is effective and improved its efficacy significantly in reducing the inflammation of acetic acid-induced colitis. Also, the biochemical tests performed suggest that this COX2 inhibitor could be further investigated for the treatment of various inflammatory bowel diseases such as ulcerative colitis or Crohn’s disease moving up to colon cancer.

References


Table 5. ANOVA results of the biochemical tests.

<table>
<thead>
<tr>
<th>Tukey’s Multiple Comparison Test</th>
<th>TNF-α level</th>
<th>PGE2 levels</th>
<th>NOS activity</th>
<th>MPO level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant difference (p&lt;0.05)</td>
<td>*</td>
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<tr>
<td>Normal control versus Acetic acid control</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Normal control versus Group 1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Normal control versus Group 2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Normal control versus Group 3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Normal control versus Group 4</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Acetic acid control versus Group 1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Acetic acid control versus Group 2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Acetic acid control versus Group 3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Acetic acid control versus Group 4</td>
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<td>*</td>
<td>*</td>
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</tr>
<tr>
<td>Group 1 versus Group 2</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Group 1 versus Group 3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Group 1 versus Group 4</td>
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<td>Group 2 versus Group 4</td>
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<td>Group 3 versus Group 4</td>
<td>n.s.</td>
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</tr>
</tbody>
</table>

Group 1 treated with celecoxib-loaded Eudragit S100 microparticles, Group 2 treated with celecoxib-loaded Eudragit L100-55 microparticles, Group 3 treated with celecoxib-loaded Eudragit L100 microparticles and group 4 treated with celecoxib suspension (+ significant, n.s. not significant).


