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Research Article

Study the potency of colonic pellet containing ibuprofen on the suppression of gastro duodenal ulceration in male Wistar rats

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ABSTRACT

In the previous study, the colonic release of ibuprofen pellet has been developed by using the microbial dependent release system which was successfully obtained by using the dual coatings. Pharmacokinetic study of this pellet had been done in New Zealand albino rabbit and showed delay absorption, but better bioavailability compared to conventional oral preparation. Therefore, the aim of this study is to explore the potential benefit of this formulation in minimizing the main side effect of ibuprofen: gastric duodenal ulcer. To study the suppression effect on ulcer *in vivo*, male Wistar rats were used and divided into 4 groups: negative control, group given ibuprofen suspension, group given uncoated ibuprofen pellet and group with coated ibuprofen pellet. All treated groups were administered orally twice a day for 14 days. Then, parameters including platelet counts and observation on gastroduodenal ulcer development both visual and histological analyses were performed. Groups receiving both suspension and uncoated pellet of ibuprofen showed ulcer development, meanwhile this condition did not occur in rats treated with coated pellet. Colonic release of ibuprofen seems to be promising in the suppression of ulceration. The efficacy of anti-inflammatory activity of the formula is now under progress.

Keywords: Colon targeting; coating pellet; ibuprofen; inulin; shellac; ulcer; microbial-dependent release; histopathology

INTRODUCTION

The colonic drug delivery system has been developed to facilitate the local effects of drugs on the colon as well as to improve drug bioavailability, which undergoes degradation in the upper gastro intestinal tract (GIT). Colonic delivery system can be performed in three different approaches based on pH (pH dependent system), transit time in each compartment (time dependent system) and based on the activity of microflora in the colon (colonic microflora activated system) (Fatima L., et al., 2006). Maximal release of active substance in colon can be achieved using a combination of the approaches and selection of excipients and manufacturing techniques with appropriate dosage.

Ibuprofen is a non steroidal anti-inflammatory drug that widely used in treating fever, inflammation, mild pain, and moderate, it is absorbed easily along the digestive tract (Gohel et al., 2009). Nonsteroidal anti-inflammatory drugs use is frequently limited by gastrointestinal side effects, ranging from dyspepsia to life-threatening bleeding from ulceration (Perez et al, 1997). It is believed that NSAIDs by inhibiting COX pathway causes inhibition of prostaglandins synthesis, which are responsible for maintaining gastric mucosal integrity (Garcia et al, 2001). Upper gastrointestinal endoscopy studies have shown a 15-30% prevalence of ulcers in the stomach of patients taking NSAIDs regularly (Gajraj, 2003). According to prospective data from Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS), 13 of every 1000 patients with rheumatoid arthritis, who take NSAIDs for one year have a serious gastrointestinal complication (Garcia et al, 2001; Singh, 2000). We developed colonic release of ibuprofen to avoid direct contact of this drug with upper part of GIT. We are wondering whether by preventing the contact may reduce or even diminish the gastroduodenal ulcer. Our formula was containing ibuprofen pellets made using a matrix cross-link alginate and guargum then double-coated with inulin-shellac combination (Rachmawati et al, 2011). This preparation is commonly used as a core coating for drug delivery systems in the colon. This construction is proven to release ibuprofen only when the pellet reaches the colon upon colonic microbes' action. Inulin helps the release the drug in the presence of microbial activity in the colon (microbial-dependent release), while controlling the release of shellac in the colon based on pH (pH-dependent release). The use of shellac as an outer layer protects the release drug against the conditions in the upper gastrointestinal tract. This was proven by our *in vitro* dissolution test. In line, the pharmacokinetic study of this pellet in New Zealand albino rabbit

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showed delayed absorption indicated with an increased of Tmax than conventional preparation of ibuprofen. Higher $AUC_{o-\sim}$ of ibuprofen coated pellet as compared to ibuprofen suspension indicates that our formula does not affect the efficiency or even better absorption of ibuprofen orally (Rachmawati et al, 2011).

Many reports described formulation of ibuprofen to release in colon. However, none of them studied *in vivo* about the potential of this colonic targeting release on preventing the main side effect of daily oral administration of ibuprofen. So, this is the first study evaluating whether by avoiding the direct contact of ibuprofen in upper part of GIT will diminish gastroduodenal ulcer. Male Wistar rat was used for this study, and the parameters observed including platelet count, visual observation by counting and measuring the ulceration spots and length of the wound occur in the mucosa lining of the upper GIT and histological analysis of the targeted organs.

MATERIALS AND METHODS

Chemicals

Ibuprofen, Avicel PH 101, Natrium alginate, Calcium acetate, Guargum, Polyvinylpyrrolidone (PVP), Inulin, Shellac were purchased from Kimia Farma, Bandung Indonesia, Polyethyleneglycol (PEG 6000, Bratachem, Bandung, Indonesia), Natrium hydroxide, Dibasic phosphate, mono basic Phosphate were purchased from Merck (Darmstad, Germany), Hematoxyline (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), Eosin (Sigma Aldrich Chemie GmbH, Steinheim, Germany), Turk solution (Sigma Aldrich Chemie GmbH, Steinheim, Germany), Ammonium oxalate (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), Formaldehyde (Bratachem, Bandung, Indonesia). All materials used were pharmaceutical grade.

Animal

Male Wistar rats weighed around 200 grams, was divided into 4 groups (each, n = 5): negative control, group given the suspension of ibuprofen, group given coated ibuprofen pellet, and group given uncoated ibuprofen pellet. The animals were kept according to the groups separately. All groups were fed twice daily following the multiple dose regiments according to body weight, which was 1.8 mg of ibuprofen/200g of rat's body weight. This dosing regimen was prolonged to 2 weeks.

The study as presented was approved by the Local Committee for Care and Use of Laboratory Animals and was performed according to strict governmental and international guidelines on animal experimentation.

Preparation of coated pellet

Coated ibuprofen pellet was prepared as previously (Rachmawati et al, 2011), i.e. extrusion-spheronization method. Briefly, the ibuprofen and the excipients were crushed into fine particles. The well crushed ibuprofen (33.33%) was mixed with Avicel PH 101 (43.33%), PVP (4%), Guargum (17.41%), Calcium acetate (0.97%), and Natrium alginate (0.97%) in a tubular mixer for 15 minutes. The granules were spheronized using spheronizer (local product) for 1100 rpm for 1 minute until the spherical mass was formed. The pellet was then dried in the oven at 60°C for ± 90 minutes and continued for evaluation. Pellets with the range size of 315-560 µm were used as the core pellet. The subsequent double layer coating was done with 10% w/v of inulin and 10% w/v of shellac solutions. Coating was done with fluid bed dryer with coating spray setting of a solution of 1 mL/min with a pressure at 1.5 bar. Drying temperature was about 55-66˚C with the speed of a blower about 6-9 scale. The longer, the weight of the pellet increased so that the blower speed will improve to remain fluidized the pellet well. Coating was done by varying weight gain of 15% of inulin and 10% of shellac.

Evaluation of pellet of ibuprofen

Evaluation of the pellet included size, size distribution, morphology, ibuprofen content, and coating uniformity was performed according to previous report (Rachmawati et al, 2011).

In vivo evaluation

Platelet counting

Before the rats were sacrificed, the blood sample was obtained to perform the platelet counting test. 1% of Ammonium oxalate in distilled water was prepared. Further on, 0.02 mL of blood sample was mixed thoroughly into a test tube with 0.38 mL of ammonium oxalate and 0.02 mL of Ethylenediaminetetraacetic acid (EDTA) with a mechanical stirrer (Vortex). One mL of the sample solution was mixed thoroughly with 1 mL of Turk solution. Twenty µL of sample solution was filled to the mark of Hemacytometer and covered with glass slide. After 10 minutes, the slide was observed under the light microscope. The total count cells were counted using following formula:

Platelet count (cells/ L) = N× (D/A) ×10×10^6

N = the total number of cell counted

D = dilution factor

A = total area counted (in $mm^{2)}$

10 = factor to calculate a volume in µl from area (mm^2)

10^6 = factor to convert count/µl to count/l

Macroscopic observation

After the rats dissected, the gastrointestinal tracts (GIT) were dissected and cleaned perfectly with saline 0.9%. GIT were observed to check any bleeding or wound occurs. The wound was observed by measuring the length or diameter of the wound using the shove.

Histological test

GIT was preserved in the formalin buffer before preceded to the histological test. The wound tissue was sliced into 5 µm thickness using microtome and soaked with the fixation solution (formaldehyde buffer) to preserve the cells. The tissue section was also embedded in paraffin wax to increase the stability and mechanical strength and to be ease to cut into thin slices. The tissue section slide was subsequently stained with Erlich Haematoxylin and Eosin and further observed under the light microscope.

RESULTS AND DISCUSSION

Our previous study reported that the best formulation in terms of dissolution profile was the formula of the coating system consisted of 15% of inulin WG (weight gain) and 10% WG of shellac. The colonic delivery system that has been developed in the previous study used the pH dependent release shown by the protection action of shellac from acidic environment of gastric, and microbial dependent release by the action of inulin layer. The pellet from this formulation showed a good physical evaluation such as shape and size, flow ability, friability, and surface morphological tested by Scanning Electron Microscope (SEM).

Table 1: Physical evaluation of coated pellet of ibuprofen

Parameter	Value
Friability (%)	0.044 ± 0.02
Apparent density	0.63 ± 0.01
Flow rate	6.94 ± 0.25
Weight Gain total (%)	38.03 ± 1.89
Ibuprofen content	71.95 ± 2.46

Table 1 shows the parameters that the pellet met a good physical characteristic. The physical appearance and analysis of surface morphology by SEM were shown in figure 1 and 2, respectively.

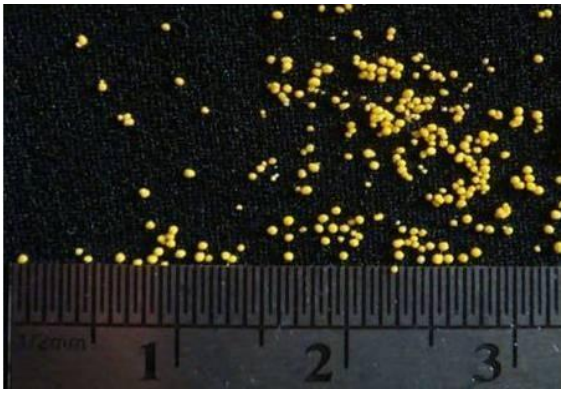

Figure 1: Physical performance of coated pellet of ibuprofen

Platelet counting

Ibuprofen is a non steroidal anti-inflammatory drug (NSAID) which is used for arthritis, fever, analgesic and also when there is an inflammatory component. It is known to have anti platelet effect where it shows a decrease in platelet aggregation, although relatively mild and somewhat short-lived, and inhibits thrombus formation. Therefore, it is effective in arterial circulation where a little effect in anticoagulant activity. From the studies, it has shown that ibuprofen released and immediately absorbed in GIT interferes the anti platelet activity. However, colonic release coated ibuprofen pellet, which was released and absorbed in colon compartment did not do so (figure 3). There is still unclear explanation but prevention release thereby avoiding direct contact of ibuprofen in upper part of GIT may suggest this effect.

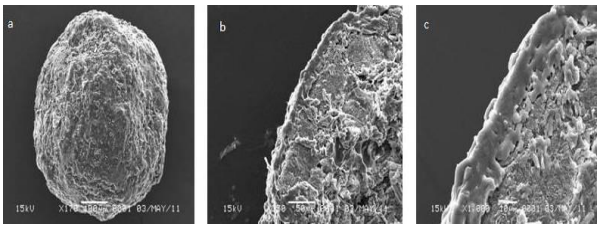

Figure 2: The surface morphology of ibuprofen pellet under Scanning Electron Microscope. a = core (uncoated) pellet (170x); b = inulin-coated pellet (450x); inulin and shellac-coated pellet (1000x)

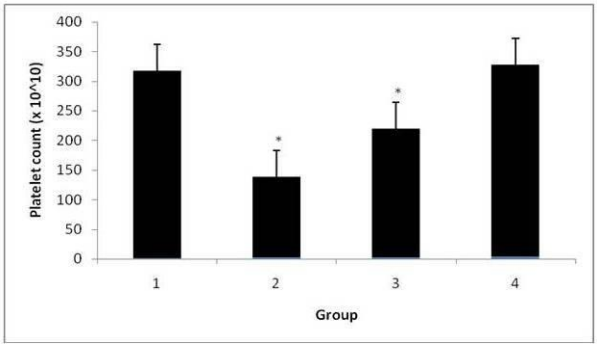

Figure 3: Number of platelets counts after the administration of Ibuprofen in various dosage forms.1 = normal group, 2= group receiving Ibuprofen suspension, 3= group receiving uncoated Ibuprofen pellet, 4= group receiving coated Ibuprofen pellet. *= p<0. 05 (as compared to the normal group)

Ibuprofen administered in suspension dosage form decreased platelet count to as low as 135.83 $x10^{10}$ from a normal value of 316.25 x 10^{10} ($p<0.005$). Coated pellet did not have any effect on the platelet count (323.33 x 10^{10}), while the uncoated pellet led to only slight decrease in the value (216.25 x 10^{10}), which was not significant statistically when compared to the normal group as well as to group receiving coated pellet of ibuprofen. Diminish anti platelet effect of ibuprofen formulated for colonic release confirms that delay release of ibuprofen until reaching colonic compartment resulted in delay absorption of ibuprofen. This in turn may lead to less effect of ibuprofen in regard to anti platelet activity.

Ulcer observation

Table 2 shows the effect of formulation on the gastro-duodenal ulcer development. As seen, ibuprofen aimed for colonic release did not show any damage both in gastric and upper part of an intestine.

Table 2: The gastric and duodenal ulcerations developed in rat after administration of the ibuprofen from different formulas

Group	Observation	
	Diameter of Spots (mm), gastric ulcer	Length of lines (mm), duodenal ulcer
1	0	0
2	1.667 ± 1.156	2.333 ± 1.528
3	1.333 ± 1.366	16.167 ± 14.469
4	0	0

1 = control; 2 = treated with ibuprofen suspension; 3 = treated with uncoated ibuprofen pellet; 4 = treated with coated ibuprofen pellet.

In contrast, an ulcer was developed when ibuprofen has chance to contact directly with the tissues where it was released (group 2 and 3). In regard to this release, clear damage was seen in group 2 and 3. A minor gastric ulcer indeed observed in group receiving uncoated pellet of ibuprofen (enteric release). As reported in our previous work, uncoated pellet of ibuprofen showed the release in gastric compartment (at first 2 h of dissolution test) accounted for nearly 20% (Rachmawati et al, 2011). This clearly explains the gastric ulcer was also occurred in this group. Similarly, duodenal ulcer represented by the length of the line was shown as well in group receiving the enteric release of ibuprofen as compared to ibuprofen suspension.

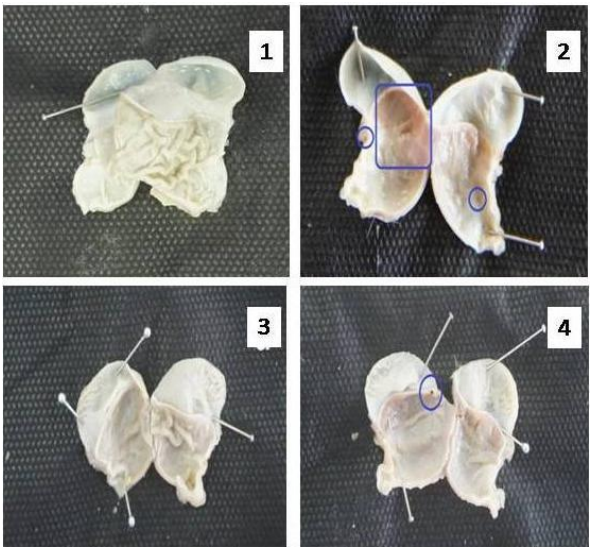

Figure 4: The macroscopic of gastric after ibuprofen administration: (1) normal group, (2) group receiving ibuprofen suspension, (3) group receiving uncoated pellet of ibuprofen and (4) group receiving coated pellet of ibuprofen. No ulcer was observed in the group treated with a coated pellet formula. The arrows indicated bleeding, circle indicated ulcer.

In line with the damage measurement, macroscopic as well as microscopic observations (figure 4 and 5) confirm the effect of release of ibuprofen on the gastro-duodenal ulcer development.

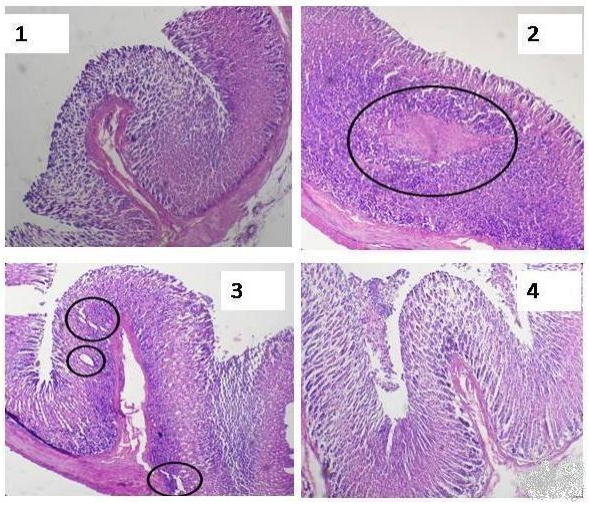

Figure 5: Microscopic observation of gastric (100x). (1) normal group, (2) group receiving ibuprofen suspension, (c) group receiving uncoated pellet ibuprofen, (d) group receiving coated pellet ibuprofen. There was a lesion in lamina propria (2, circle) and lamina propria damage (3, circle)

As shown in figure 6, it is clearly seen that group receiving uncoated ibuprofen pellet developed most duodenal ulcer significantly as compared to the normal group.

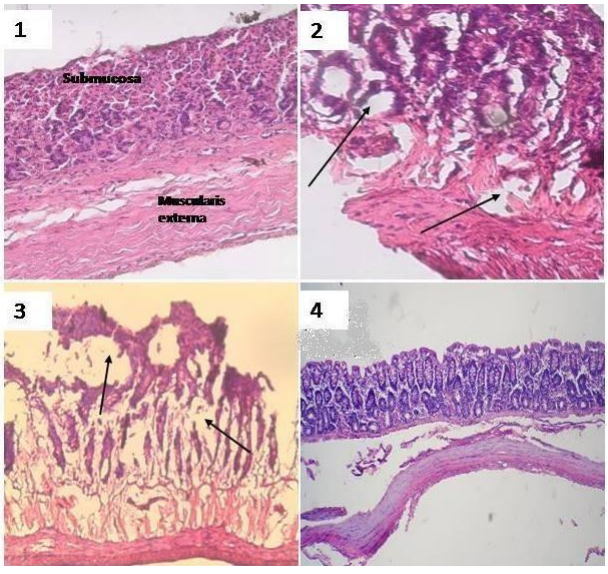

Figure 6: The duodenal lining of the rats (arrows) observed under the light microscope

Duodenum is the closest segment to the stomach. This portion of the small intestine receives signals from the stomach and digestive secretions from the pancreas

and liver and neutralizes its acid before they can damage the absorptive surfaces of the small intestine.

This is relevant with the direct contact of ibuprofen to the compartment where it was released. In uncoated pellet, the release of ibuprofen was more pronounce occurred in the intestinal region suspension. Whereas, group receiving ibuprofen suspension showed ulceration more in the gastric though a minor ulcer was also developed in the upper part of an intestine. In contrast, group given coated pellet of ibuprofen did not develop any ulcer both in gastric as well as in an intestine. The avoidance of ibuprofen release in these compartments clearly explained the absence of the ulcers.

For the histological test, major layers of digestive tract were observed to include the mucosa, sub-mucosa, muscularis externa, and serosa. As shown in figure 5 and 6, administration of ibuprofen suspension and uncoated pellet showed a massive breakage of cells compare to the normal group. These have been shown by the damage of gastric pit and mucous epithelium. The lamina propria was unavailable to be seen at all. The sub mucosa cell was eroded where the cell in the layer was not fully compacted as the normal group and coated ibuprofen pellet (figure 6). Therefore, it was shown clearly that the release of ibuprofen from the suspension and uncoated pellet dosage forms in the upper GIT damaged the tissues, suggested due to the action of higher acidity cyclo-oxygenase reaction. As well known, ibuprofen is a nonselective COX inhibitor, in that it inhibits two isoforms of cyclooxygenase, COX-1 and COX-2. The analgesic, antipyretic, and anti-inflammatory activity of NSAIDs appears to operate mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on the gastrointestinal tract (Rao and Knaus, 2008). However, the role of the individual COX isoforms in the analgesic, anti-inflammatory, and gastric damage effects of NSAIDs is uncertain and different compounds cause different degrees of analgesia and gastric damage (Kakuta, 2008).

The gastric ulcer normally continued in duodenal phase. From the figure above, an erosion of submucosa layer and slight erosion of the muscularis externa layer can be seen in figure 6(2) and 6(3), meanwhile figure 6(4) is similar with the normal group.

CONCLUSION

Administration of ibuprofen formulated in colonic release coated pellet surprisingly showed similarity in parameters observed to normal indicating no gastro-duodenal ulcer was developed, in contrast with gastric as well as the enteric release of ibuprofen. This result indicates that development of coated pellet offering microbial-dependent release of ibuprofen seems to be promising in the suppression of the main side effects of ibuprofen, i.e. ulceration. Further study to confirm and to explain the mechanism of ulceration inhibition needs to be carried out.

AUTHOR'S CONTRIBUTION

HR and KA have made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data. HR has been involved in drafting the manuscript or revising it critically for important intellectual content. K Apparavo and A have contributed in animal work, histological work, microscopic and macroscopic observations. K Apparavoo has been involved in the formulation and characterization of the preparation. All authors read and approved the final manuscript.

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