NOVEL PROTECTIVE ROLE OF MONTELUKAST AGAINST ASPIRIN INDUCED GASTRIC ULCERATION IN RABBITS - MONTELUKAST; A REVOLUTIONARY ASPECT

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ABSTRACT

Objective: To assess the gastroprotective effect of montelukast on aspirin induced gastric mucosal ulceration in rabbits.

Study Design: Laboratory based quasi experimental study.

Place and Duration of Study: National Institute of Health Islamabad and department of Pharmacology and Therapeutics in collaboration with Histopathology department at Army Medical College Rawalpindi from March to May 2018.

Methodology: Twenty eight New Zealand white rabbits were randomly divided into four groups as follows: Group 1 was normal control group, group 2 received aspirin as a single dose of 300mg/kg orally on day 6 of the study, group 3 and 4 received omeprazole and montelukast respectively on a similar dosing schedule: where a total of 10mg/kg/day of each respective drug was administered orally for 6 consecutive days followed by a single oral dose of 300 mg/kg of aspirin after 1 hour of the last dose. All animals were sacrificed 5 hours after aspirin administration. The abdomens were dissected and the gastric tissues were excised for macroscopic, ulcer index and pH evaluation and were sent for histopathology for microscopic assessment.

Results: Moderate to severe gastric mucosal damage (grade II-III) was seen in the aspirin treated toxic group, with considerable increase in ulcer index and decrease in pH (p<0.001). Montelukast significantly ameliorated the gastric mucosal ulceration as evident by substantial decrease in ulcer index (UI), increase in pH, gross and histoarchitecture findings with preventive index of 51.36% closed to the percentage protection of standard anti-ulcer drug omeprazole.

Conclusions: Montelukast has anti-ulcerogenic gastroprotective effect on microscopic, macroscopic and biochemical level.

Keywords: Aspirin, Gastroprotection, Montelukast, Omeprazole, Peptic ulcer disease.

INTRODUCTION

Peptic ulcer disease is one of the most prevailing multifactorial disorders of gastro-intestinal system[1]. World Health Organization reports that annually almost 8-10% of the population is affected by peptic ulcer disease in their life[2]. In quest of major causative factors of peptic ulcer disease, it is eminent that epidemiologically there is existence of appreciable relationship between non-steroidal anti-inflammatory drugs (NSAIDs) and peptic ulcer disease[3]. Until now, aspirin is the most extensively used NSAIDs with universally acclaimed anti-inflammatory and anti-platelet properties[4]. However, in current era, its long term use in low doses is associated with gastric mucosal injury and is a matter of great clinical interest as it restricts the aspirin's clinical efficacy.

Aspirin is included in one of those NSAIDs that cause direct topical injury to gastric epithelial cells resulting in detrimental outcome in the form of significant gastric ulceration. The precise ulcerogenic mechanisms of aspirin induced gastrointestinal toxicity are direct topical damage, cyclooxygenase enzyme inhibition diminishing gastroprotective prostaglandins, liberation of pro-inflammatory mediators and reactive oxygen species[5]. Approximately 40% of low dose aspirin

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consumers acquired gastrointestinal complications and are thereby remain a crucial challenge for health professionals[6].

Montelukast is a universally renowned anti-leukotriene cytoprotective agent with a phenomenal safety profile, wide therapeutic index and no tolerability issues making it extremely useful in respiratory ailments[7]. Montelukast has a noteworthy capability to impede ROS production and undesirable inflammatory mediators release by causing blockage of cysteinyl leukotriene receptor (CysLTR). It competitively blocks leukotriene D4 (LTD4), thereby prevents its binding and thus producing its bronchodilatory effects on respiratory airways[8]. Hence, on the basis of aforementioned property of it, this study was aimed to explore novel, effective and safer gastroprotective therapy against prolong use of low dose aspirin to alleviate chronic gastropathy, lower medications burden and improve patient compliance in comorbid conditions when they are used in combination regimen.

METHODOLOGY

This study was accomplished in the National Institute of Health (NIH) Islamabad and in the Department of Pharmacology and Therapeutics in association with Department of Histopathology at Army Medical College (AMC) Rawalpindi after obtaining approval by ethical review committee of AMC. By non-probability consecutive sampling technique and stratified randomization method, twenty eight New Zealand white rabbits of both gender (twenty males and eight females excluding pregnant females), aged between 3 and 4 months, weighing around 1-2 kg, were selected for this quasi experimental study. Standard laboratory conditions and commercial diet including peas, carrots, and grams with free tap water access ad libitum were provided in the animal house of NIH. The study was conducted for six days. Animals were randomly designated in to four groups, each group comprising of seven rabbits. All rabbits were kept fasted for forty eight hours before aspirin dose administration but with free availability of water. Earlier to the intervention, rabbits were weighed for precise modification of dose. Animals (n=7) in each specific group were administered drug according to following intervention protocol. Groups 1 served as the normal control group and remain untreated during whole experiment. Group 2 was aspirin control group (toxic group), received a single oral dose of aspirin 300mg/kg[9] on 6th day of the experimental study. Group 3 received omeprazole 10mg/kg[10] orally for 6 days together with aspirin administration one hour after omeprazole on day[6]. Group 4 received montelukast 10mg/kg[11] orally for 6 days with aspirin one hour after montelukast administration on day 6. All drugs were given at the same time to prevent diurnal variation of stomach secretions regulators. After five hours of the single ulcerogenic dose of aspirin, rabbits were euthanized and stomachs were excised out. Its luminal contents were drained in to jar for assessment of pH by using pH meter. Then washed and examined grossly by using magnifier hand lens to find out ulcer index (U.I). To ascertain the degree of ulcerations and later the calculation of an ulcer index, formula used was:

$$\text{Ulcer index (UI)} = UN + US + (UP \times 10^{-1})$$[12]

UI = ulcer index, UN = ulcers on average in an animal, US = severity score on average per animal[13], UP = animals with ulcer on percentage. Ulcers in stomach were measured in millimeters and 01 millimeter of ulcer was equal to 05 petechial lesions per stomach[14].

The preventive index for every group was determined by using following formula

$$\text{Preventive index (\%)} = [(UI_{CONTROL} - UI_{TREATED} / UI_{CONTROL})] \times 100$$[14]

Afterwards the glandular stomach was fixed in 10% neutral buffered formaline for histological cross examination. After fixation, gastric tissue was processed for dehydration with ethyl alcohol and then they were subjected to clearing, paraffin embedding and sectioning. Almost 4-5 micrometer thickened portions were mounted and stained with Hematoxylin and Eosin and

evaluated for histopathological gastric tissue injury according to grading criteria of Dixon's scoring system[15] which includes foveolar hyperplasia, vascular congestion, mucosal edema and smooth muscle fibers in lamina propria.

The data related to ulcer index and pH was shown as mean ± SD and analyzed by SPSS version 22. For comparison of parameters one way analysis of variance (ANOVA) was used. Application of Post Hoc Tuckey test was done to validate significance of difference between two observations. The histopathological results were analyzed with the help of Chi Square test. *p*-value ≤0.05 was studied as significant between observations.

RESULTS

A total of 25 white rabbits, 20 male and 8 were females, group 2 (aspirin treated group) showed a highly significant rise in ulcer index 14.5 ± 0.71 alongside remarkable decrease in pH (2.4 ± 0.04) as compared to the normal control group (*p*-value <0.001). Pretreatment with omeprazole and montelukast depicted hugely comparable decrease in ulcer index and increase in pH values (*p*-value <0.001) with percentage protection (preventive index) of 54% and 51% respectively when comparison with aspirin treated group was done as given under table and fig-1 & 2. Though omeprazole exhibited more preventive index than montelukast but the differences between them were statistically insignificant (fig-2). The mean ± SD along with *p*-values of gastric U.I and pH of all the groups are mentioned in table.

Statistical analysis was carried out by one-way ANOVA followed by Post Hoc Tuckey Multiple Comparison Test.

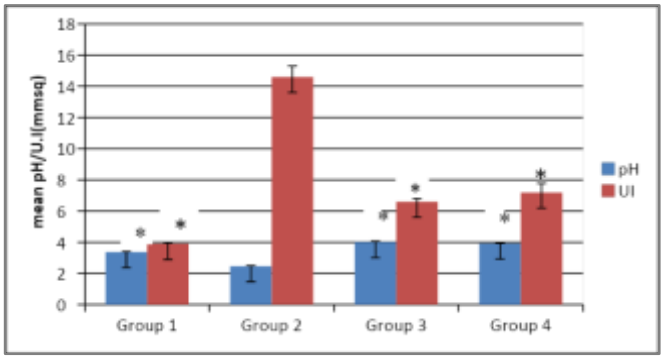
a = $p \leq 0.05$ compared with the control group

b = $p \leq 0.05$ compared with the aspirin control group

Table: Comparison of effects of understudy drugs upon gastric pH, U.I and preventive index of New Zealand white rabbits.

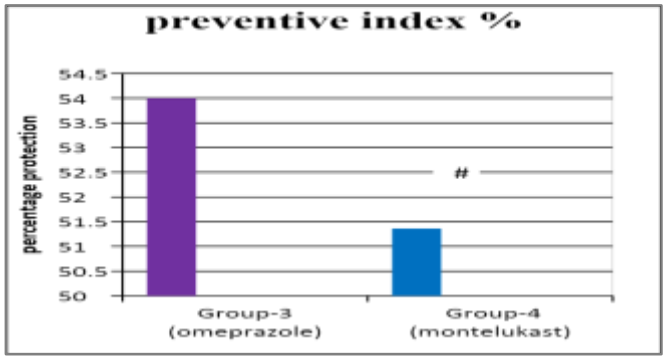
GROUPS	pH	U.I (mm²)	Preventive index
	Mean ± SD	Mean ± SD	
Group-I (Control group)	3.3 ± 0.04	3.9 ± 0.05	-
Group-II (Aspirin control)	2.4a ± 0.04	14.5a ± 0.7	0
Group-III (Omeprazole + Aspirin)	4.0b ± 0.06	6.6b ± 0.2	54
Group-IV (montelukast + Aspirin)	3.9c,d ± 0.02	7.1c,d ± 0.6	51
p-value	≤0.001*	≤0.001*	

*significant between groups



*Indicate significant change from aspirin control group with $p \leq 0.05$ using ANOVA followed by post hoc Tuckey test

Figure-1: Effect of studied drugs (aspirin, omeprazole and montelukast) on gastric pH and U.I of rabbits. Data represents mean ± S.E.M (n=7).



#Indicates insignificant change with $p \geq 0.05$

Figure-2: The effect of protective drugs on gastric preventive index of rabbits.

c = $p \leq 0.05$ compared with the aspirin control group

d = $p \geq 0.05$ compared with the omeprazole + aspirin group

On macroscopic examination, the stomachs from group 2 (aspirin treated group) revealed severe gastric mucosal tissue injury with multiple petechial spots and hemorrhagic lines as compared to normal control and omeprazole pretreated group both of which showed almost normal gastric architecture (fig-3a, 3b). On comparison with toxic group significant gastroprotection was observed with montelukast pretreated group with nearby normal stomach tissue architecture without evidence of any sub mucosal hemorrhagic lesions and ulcers (fig-3c).

Histopathological assessment was done under light microscopy in a blinded fashion, the stomachs from the aspirin treated toxic rabbits illustrated moderate to severe gastric mucosal injury (grade II-III) according to Dixon's score of chemical gastritis[15] showing foveolar hyperplasia, striking mucosal edema and hyperchromasia (fig-4a). Normal control and omeprazole pretreated group fell into grade 0 i.e normal gastric tissue histological architecture (fig-5a). Montelukast pretreatment successfully abated aspirin-induced foveolar hyperplasia and many other histopathological changes of chemical gastritis (fig-5b). Hence, slides from montelukast pre-treated animals were graded to be normal (grade 0)[15]. On applying chi-square test on all the values of experimental sets the results were statistically significant ($p<0.05$).

DISCUSSION

The unwanted consequences of co-administration of various drugs on human well- being have become censorious issue for physicians at this time as numerous patients are on combination regimen at the same time. Gastric mucosal ulcers related with aspirin and other NSAIDs are primary dilemma of public health[16]. In this study, acute gastrointestinal toxicity by a single dose of aspirin was generated. Numerous underlying mechanisms leading to aspirin-induced gastropathy have been explained in previous studies. Direct local injury, reduction of gastroprotective prostaglandin synthesis owing to irreversible cyclooxygenase enzyme inhibition, generation of pro-inflammatory leukotrienes due to shunting of arachidonic acid metabolism from cyclooxy-

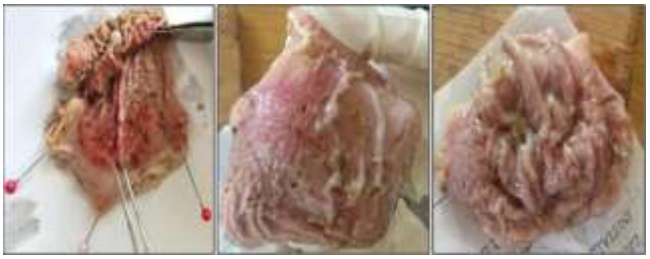


Figure-3: Gastroprotective effect of daily administration of montelukast and omeprazole on gross changes induced by aspirin in the gastric tissues of rabbits. Photograph (A); significant hemorrhagic streaks with gastric mucosal ulceration (group-2). Photograph (B); near normal gastric tissue histoarchitecture (group3). Photograph (C); nearly normal gastric tissue morphology (group-4).

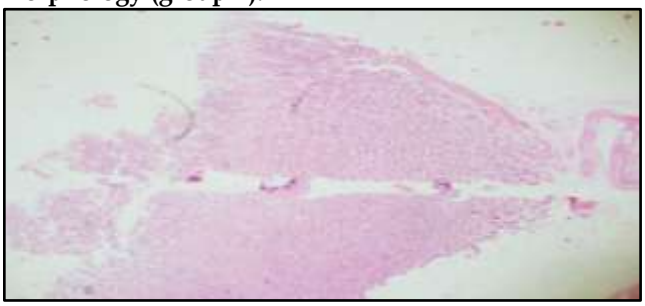


Figure-4: Gastro-ulcerative effect of single dose aspirin administration on stomach histoarchitecture. Photomicrographs of gastric tissue of aspirin treated group stained with hematoxylin and eosin showed foveolar hyperplasia x 10 associated with hyperchromasia with mitotic figures and mucosal edema (a).

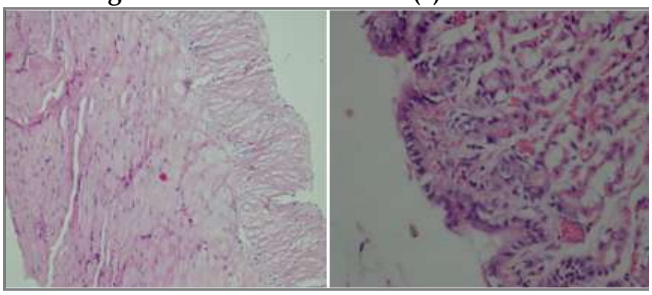


Figure-5: Gastroprotective effect of daily administration of omeprazole and montelukast on histopathological changes induced by aspirin in the gastric tissue of rabbits. Photomicrograph (A); intact gastric tissue mucosal layer x 10 (group-3). Photomicrograph (B); intact epithelium of gastric tissue without foveolar hyperplasia x 40 (group- 4).

genase to lipoxygenase pathway which was later accompanied by inflammation and generation of oxygen derived free radicals are considered to be the hallmark of aspirin induced gastric ulceration[17-19]. Considering central role of leukotriene as well as oxygen derived free radicals in aspirin induced gastric mucosal injury we decided to introduce novel gastroprotective effect of montelukast for its attenuation when they are used in combination with aspirin in co-morbidities.

Aspirin control group was taken a single ulcerative dose of aspirin after 48 hours of fasting on sixth day of experimental study. This group has proven noticeable gastric tissue injury corresponded with remarkable elevation in ulcer index (p-value <0.001) and reduction in pH (p-value <0.001) when compared with normal control group. Such devastating results were validated and claimed by El-Ghaffar and many previous researchers[20-22]. Gross and histo-pathological damage were considered to be moderate to severe (grade III) and manifested as pathogonomic hall marks of acute chemical gastritis which appeared to be statistically significant on comparison with normal control. Such histological disruption was in agreement with the findings of Ali Hassan Ijam and Edwadh who previously cited such damage with high dose of aspirin[4].

Omeprazole being famous proton pump inhibitor was used as a standard reference drug in our study. All the parameters of this group showed highly significant results (p-value<0.001) in comparison with aspirin control group with preventive index estimated to be 54%. These significant outcomes were in consonance with similar strings of experiments, in which standard omeprazole was in use as well[2,23].

Montelukast is a powerful inflammatory scavenger which is receiving immense recognition for its anti-leukotriene capability. Montelukast depicted its gastroprotective potential once administered orally for six consecutive days prior to the ulcerogenic aspirin challenge as evident by gross morphology and histological findings. On comparison with the aspirin control group significant gastroprotection was observed with montelukast pretreated groups which were obvious from surprising decrease in ulcer index (p-value<0.001), increase in pH (p-value<0.001), as well as amelioration of gross and histopathological damage with near normal gastric histological architecture. All these observations along with preventive index of 51% were strikingly insignificant on comparison with standard reference drug group omeprazole. Aforementioned results explained that conse-cutive six days administration of montelukast helped resist the inflammatory markers and thus restore gastric tissue histoarchitecture. This outcome was in absolute agreement with Ozbakis and colleagues who demonstrated anti-inflammatory gastroprotective mechanism of montelukast on indomethacin induced ulcer in their work[24]. Anti-leukotriene property of montelukast against aspirin induced cyclooxygenase inhibition[11] clearly explained its mechanism of gastroprotection and our aforementioned protective findings were in concurrence with study conducted in 2017 by risk and co-workers in which montelukast was proclaimed to improve dexamethasone induce gastric damage in experimental rats model. Our findings that montelukast has promising anti-ulcerative effect were consistent with some previous works[4,14,25].

However, the this study was revolutionary in this regard that highly popular montelukast was previously least explored against aspirin in terms of its gastroprotective effect. Thus this study successfully presents that cytoprotective montelukast pretreatment remarkably alleviate marks of aspirin induced gastritis. This will further lessen demand of omeprazole which have many detrimental effects on long term use. Hence, this study will also aid in improving patient compliance and decrease medication burden in co-morbidities.

CONCLUSION

Outcomes of our project clearly illustrated the gastroprotection of montelukast against

aspirin induced gastrointestinal ulceration. Montelukast was a powerful inflammatory scavenger. Although levels of leukotrienes in our recent project were not investigated but the amelioration of aspirin induced gastric injury by leukotriene receptor antagonist montelukast possibly interpreted their function in producing pathogonomic hallmarks of chemical gastritis. Thus, protective effect of montelukast can be promising for patients with respiratory ailments who use aspirin in low doses for their co-morbidities.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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