COMPARING THE ULCEROPROTECTIVE EFFECTS OF VERAPAMIL, RANITIDINE COMBINATION WITH OMEPRAZOLEON ASPIRIN INDUCED GASTROPATHY IN RABBITS

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ABSTRACT

Objective: To evaluate the ulceroprotective effects of Verapamil on aspirin-induced gastropathy in rabbits.
Study Design: Laboratory based, quasi-experimental study.
Place and Duration of Study: The study was conducted at the department of Pharmacology and Therapeutics in collaboration with Clinico-Pathologic Laboratory, Army Medical College, Rawalpindi, from Apr to Jun 2016.
Methodology: Eighteen rabbits were split into three groups, each consisting of six rabbits. Weight of rabbits was measured and dose was calculated accordingly. The first group was given single ulcer producing dose of aspirin 150mg/ kg body weight orally, after an overnight fast. The protective group was administered oral Verapamil 08mg/kg of body weight. Rabbits were sacrificed 01 hour after ulcerogenic dose of aspirin. Stomachs were recovered for ulcer index, pH and were referred for histopathology. Statistical analysis was carried out by using Microsoft Office Excel 2013 and SPSS version 22. One-way ANOVA, followed by 'Post Hoc Tukey' test was used for biochemical parameters.
Results: Significant gastropathy was seen in toxic groups, with substantial elevation in ulcer index (p=0.001) and distinctly low pH (p=0.001). Ulcer protection was moderate with verapamil groups (percentage protection calculated for Verapamil was 15.18%).
Conclusion: The ulcerogenic activity of Ca^{2+} influx (connected to peptic acid and gastrin secretion) was found to be ameliorated by antagonistic action of verapamil.

Keywords: Aspirin, Gastropathy, Ulceroprotective, Verapamil.

INTRODUCTION

Acid Peptic Disease (APD) is a common, multi-factorial disorder with intricate patho-genesis of upper gastrointestinal tract. Peptic ulcers emerge when gastric acid erodes the mucosal lining of the digestive system predo-minantly stomach and duodenal mucosa. Advent of peptic ulcer is a consequence of imbalance of gastric defensive/restitution mechanisms and deleterious offensive factors[1].

Aspirin including anti-platelet low dose aspirin (81–325 mg/day), is one of the leading cause of chronic gastropathy induced by NSAIDs worldwide, where prevalence of *H. pylori* infection is declining[2,3].

Several studies have established the acid stimulating ability of calcium ions. There are reports which link conditions with elevated levels of plasma calcium such as hyperparathyroidism, hypercalcemia to increased incidence of gastric acid secretion and peptic ulcer. Calcium is a potent stimulus for gastric secretion in patients with gastrin secreting tumors; this effect is due to its ability to potentiate the response to gastrin and stimulating the release of gastrin from non-beta cell tumours[4].

Verapamil was selected as lesser amount of work had been done previously with it as opposed to nifedipine, amlodipine and diltiazem. Moreover, verapamil is postulated to have ulceroprotective properties through unknown additional mechanisms apart from its calcium channel blocking properties[5].

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The objective of this study was to assess the extent of aspirin induced gastropathy in rabbits and to explore the gastric ulcer prevention offered by verapamil as an ulceroprotective against aspirin-induced gastropathy.

METHODOLOGY

Quasi experimantal study was conducted in the department of Pharmacology and Therapeutics in collaboration with Clinico-Pathological Laboratory, Army Medical College, Rawalpindi. Eighteen healthy adult male and non-pregnant female rabbits of mixed breed, weighing 1 to 2 kg were obtained from the local market by non-probability purposive sampling. Rabbits younger than six months were excluded from the study. Standard laboratory conditions includingan average temperature of 24°C and 12 hours light and dark cycle were maintained in the animal house. Before initiation of study rabbits were acclimatized for one week[6]. Rabbits were given same standard diet consisting of carrots, turnips, peas, grams and tap water ad libitum for drinking.

Rabbits were randomly assorted into three groups, each consisting of six animals by lottery method. Both the drugs, aspirin and verapamil, were administered orally. Fresh solutions of drugs were prepared for oral administration for each animal by dissolving 150mg of Aspirinin 2 ml of plain water while verapamil 40mg was diluted in 5ml of plain water to get the required strength of 08 mg/kg of body weight. The rabbits (n=6) in distinct experimental groups were given drugs according to the following plan: Group-A was the control group and received 2 ml of distilled water orally. It was housed along with other groups under similar conditions for a similar period of time. Stomach and stomach contents were preserved for gross & microscopic examination and estimation of pH[7]. Group-B received aspirin in a single dose, orally, 150mg/kg of body weight after an overnight fast. Animals were sacrificed after 01 hour. Like in group-A[8], sample collection was composed. Group-C received pretreatment of verapamil 08 mg / kg once orally, one and a half hour before aspirin exposure and sample collection was carried out as in group-A[8].

All the animals were sacrificed 01 hour after the ulcerogenic dose of aspirin, a cut was given on the ventral side of neck with a sharp knife leading to frank bleeding from the carotid vessels. The abdomen was opened by midline longitudinal incision after death of the animal, the xiphoid process was removed and the stomach was completely exposed. The stomach was taken out after dissecting all the attachments and placed in a petri dish. Each stomach was incised along the greater curvature; its contents were drained quickly and were completely recovered by washing with 10 ml of saline. Each stomach was pinned flat on white paraffin wax-filled petri dish and examined for ulcers by means of a hand lens (x10) to determine the ulcer index as described below[5]. Successively, the whole stomach was preserved in 10% formalin for histopathological examination.

Macroscopic Examination: (i)Determination of ulcer index (U.I) - Ulcer index was estimated by gross examination using a hand lens. The number and severity of injury was quantified by the following formula.UI = Un + Us + (Up×10-1). WhereUn = average number of ulcers per animal, Us = average severity score, Up-1 = percentage of animals with ulcers[9].(ii) Calculation of number of ulcers per stomach - Ulcers induced by aspirin were heterogeneous or polymorphic. Stomach was examined for number of ulcers. Linear ulcer was expressed in millimeters and 05 petechiae were calculated as 01 mm of ulcer[10]. (iii) Ulcer severity score criteria: Peptic ulcer severity scoring is as under: 0 = no ulcers, 1 = mucosal changes limited to superficial layer with no hyperemia, 2 = half the thickness of mucosa was showing necrotic changes, 3 = 2/3rd or more than that of the mucosal thickness was showing necrotic changes, 4 = total destruction of the mucosa with hemorrhage[5].(iv) Percentage protection was calculated by this formula = (C-T/C) × 100Where: C= ulcer index in control group, T= ulcer index in treated group[11].

Procedure of measurement of pH: The contents of each stomach were removed, mixed and the pH was determined using a pre-calibrated pH211 Microprocessor pH Meter (Hanna Instruments). pH measurements were taken a total of three times with the gastric contents being re-mixed, the pH meter was being washed with distilled water (having neutral pH) and the calibration checked between the measurements. HI 1333 probe was used having a spherical tip (diameter 7.5 mm); it was ensured that the homogeneous mixture of gastric contents covered the probe tip, and a stable reading be attained. The order in which the pH of the different gastric contents was read was varied within each group to abate any influence of post-sacrifice time on pH[12].

Histological sections of the stomach (antrum and pyloric areas) from all the rabbits were qualitatively and quantitatively assed, and subsequently scored according to modified version of Dixon's Systemcriteria[13]. Negative = 0, Mild=1, Moderate=2 and Severe=3[13].

The data regarding ulcer index was expressed as means ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) trailed by Tukey post hoc test for multiple comparisons. A *p*-value ≤0.05 was considered as statistically significant.

RESULTS

Table: The comparison of U.I & pH of Group A, Group B and Group C.

Parameters	Gp-A (Control) (n=6)	Gp-B (Aspirin) (n=6)	Gp-C (Verapamil) (n=6)
U.I	3.96	13.83	11.73
Mean ± SD	± 0.97	± 0.79	± 0.63
p-value		0.001	
pH	3.83	2.92	3.18
Mean ± SD	±0.04	±0.13	± 0.56
p-value		0.001	
% Protection			15.18

Total 18 rabbits were selected and randomly divided into three groups equally.There was a significant difference in mean U.I values when group-A (control) was compared with group-B (Aspirin alone), 3.96 ± 0.97 and 13.83 ± 0.79 respectively with *p*=0.001. pH showed a similar tendency and the readings were mean 3.83 ± 0.04 for control group and mean 2.92 ± 0.13 for group B with *p*=0.001.

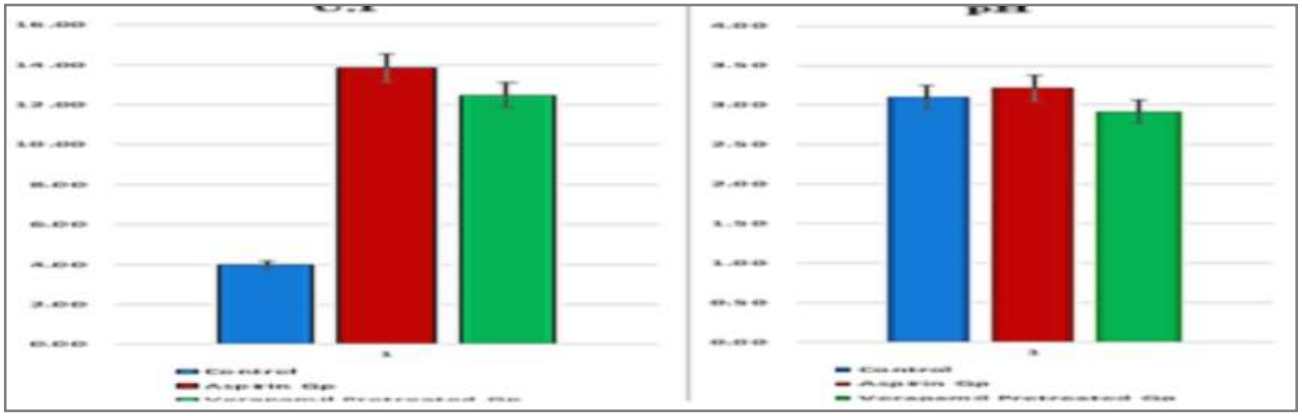

Figure-1: Comparison of U.I & pH of Gp-A, Gp-B & Gp-C, *p*-value<0.05.

Group-B (Aspirin) when compared with group-C the mean U.I values 13.83 ± 0.79 and

11.73 ± 0.63 respectively with p<0.001. pH of group-B was mean 2.92 ± 0.13 and Group-C was mean 3.18 ± 0.56, on comparison the pvalue was computed to be 0.01. Ap-values were also significant between group A and C in U.I and pH as p<0.001 and p=0.02 respectively. Percentage protection offered by verapamil was 15.18% (table).

The histopathology revealed parallel picture when group-A & B were compared. Aspirin treated group showed severe (grade-3) chemical gastritis (fig-2), and the difference between the two groups was statistically significant with a value of p=0.001. On the contrary,verapamil pretreated group yielded mild to moderate chemical gastritis (grade 1 to 2 respectively) fig-2, which evidently showed ulceroprotective role of verapamil. The difference between the two groups was statistically significant with a value of p<0.001.

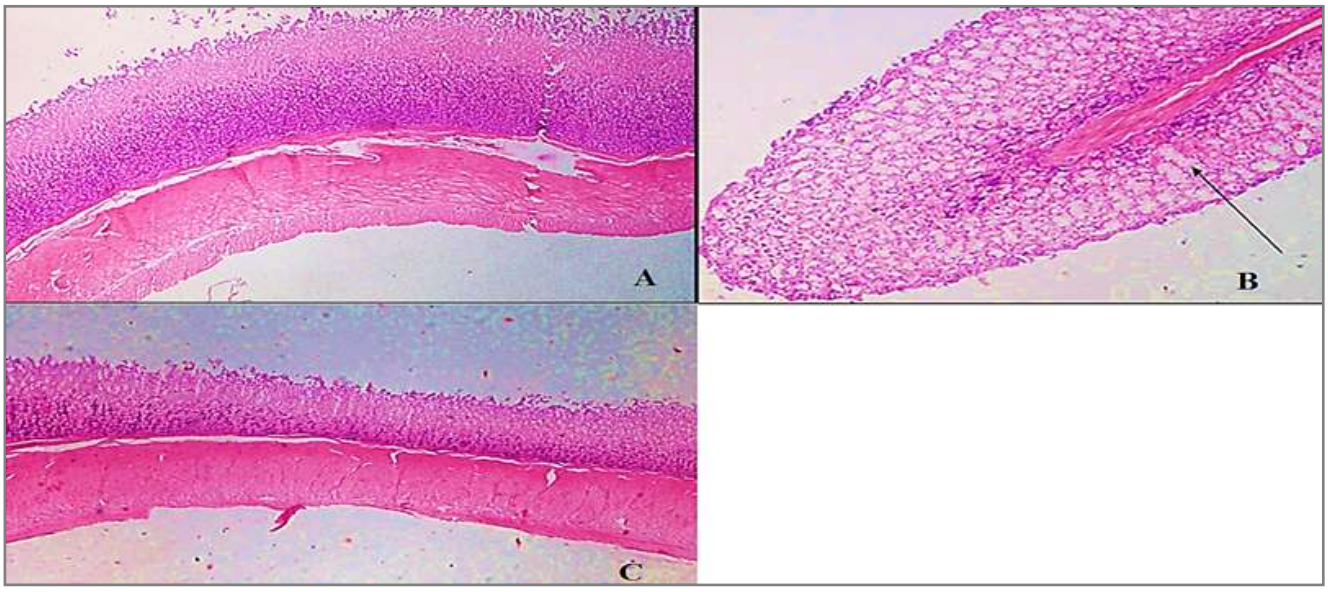

Figure-2: Essentially normal gastric mucosa (group-A) x10. (B) shows marked mucosal edema (black arrow) ×40, (Rt. upper) ×100. (group-B), In 'group-C' there is slight mucosal edema ×10, with restoration of gastric mucosa.

DISCUSSION

In this study, carried out on rabbits, aspirin was used to induce mucosal injury as it is the most frequently used NSAID, and has a propensity to produce APD even in low doses where it is employed as an anti-platelet agent[2,14].An ulcerogenic dose of aspirin was determined by conducting a pilot project in light of previous work[7] carried out on the topic. Eventually, a dose of 150mg/kg body weightreliably produced anticipated mucosal damage in the test animals and was selected for our study. Out of the calcium channel blockers, verapamil was prudently chosen as lesser amount of work had been done previously with it as opposed to nifedipine, amlodipine and diltiazem. What is more, verapamil is postulated to have ulceroprotective properties through unknown additional mechanisms apart from its calcium channel blocking properties[5].

In this study, group-A, acted as a control and was kept in similar conditions in animal house. U.I, the determinant of gross examination, essentially revealed normal gastric mucosa with little sign of injury as expected. pH also was within the realm of normalcy. On histopathology the findings in all animals were also unremarkable.

Group-B which received a single ulcerogenic dose of aspirin after an overnight fast showed a

substantial change in U.I and pH. The findings of these three parameters were highly significant when compared with GroupA with a *p*-value <0.05. Likewise, the histopathological damage was also significant in this group on comparison with group-A. These findings are consistent with many of the other studies conducted on this topic. A study carried out on rats on gross examination exemplified by U.I. gave a mean value of 13.16 ± 0.289 quite similar to our values but in this work the dose of aspirin used was 40mg/day for 09 days orally[15]. Nevertheless, in that work pH was not measured. In another project gastric injury was produced by using a higher single dose of aspirin that is 200 mg/kg of b.w and U.I value estimated was 3.0 ± 0.002. This anomaly was due to the fact that U.I was calculated through a formula which is different from our work. Despite the different value of U.I, dose of aspirin (200mg/kg of b.w) and species of animal used, the estimated mean pH value in this study was close to our mean pH value of 2.92 ± 0.13.

Group-C was given a higher dose of verapamil prior to aspirin exposure and a significant change in U.I and pH was witnessed as compared to group-B. Likewise, the comparison of histopathological findings between the two above mentioned groups were significant. The percentage protection in this group was 15.18%. Calcium channel blockers like verapamil have produced mixed results in various studies where they have been used for ulcer protection and even where protection has been seen, the effect is mostly marginal[16]. In our study the gross findings produced a positive result likewise the microscopic picture had depicted the comparable trend. Additionally, in studies where this protective effect has been visualized the dose used of verapamil is much higher than the normal therapeutic dose i.e. up to 25mg/kg of body weight[5]. Even in the present project a dose of 8mg/kg of body weight employed, is towards the higher side and serious consideration should be given to the tolerability of such a dose in human beings[17,18].

Despite of being available in enteric coated low dose aspirin (75-150 mg), it is still liable to produce gastric mucosal damage when used over a long period of time. Omeprazole remain the mainstay. Serious concerns have risen regarding the prolonged use of omeprazole like vitamin B12 deficiency, Fe^{+2}, Ca^{+2} & Mg^{+2} ionic disturbances, high risk of enteric & pulmonary infections in bed ridden patients, gastric carcinoid tumor and rebound hyperacidity on proton pump inhibitors (PPIs) withdrawal.

This study may lead us to a viable substitute for the protection against aspirin and other NSAIDs induced gastric injuries in a select group of patients. Calcium channel blockers like verapamil have ability to impede gastric damage as shown by this study and many other similar works. Middle aged and elderly patients are being recommended these drugs for various cardiovascular problems and they have to remain on these agents for virtually the rest of their lives. This is the same age group which requires the long term use of Aspirin for conditions like Ischemic heart disease (IHD) etc. or other NSAIDs for a multiplicity of inflammatory conditions. In this set of patients chances of developing APD are genuine and it is this group which is prescribed PPIs for its prevention on a long term basis. Our work has demonstrated that as a substitute of prescribing Omeprazole like drugs, in such circumstances, a simple addition of verapamil may prove to be equally effective and safer for this purpose.

CONCLUSION

Aspirin in a single dose of 150 mg/kg body weight can be employed to induce gastric mucosal damage in a rabbit model. Verapamil has a moderate protective action in such situations in a dose 08mg/kg body weightin rabbits. Verapamil in above mentioned doses can guard against Aspirin induced APD in rabbits.

CONFLICT OF INEREST

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

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