ORIGINAL ARTICLES

RANITIDINE POTENTIATES THE EFFECT OF METOCLOPRAMIDE ON GASTRO-INTESTINAL MOTILITY IN RABBITS

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ABSTRACT

Objective: To explore the synergistic potential of ranitidine on prokinetic activity of metoclopramide on isolated duodenal model of rabbit.

Study Design: Laboratory based randomized controlled trial.

Place and Duration of Study: Multi-disciplinary Laboratory, Army Medical College, Rawalpindi with study duration of 12 months.

Material and Methods: Dose response curve of ranitidine and metoclopramide were constructed by adding cumulatively increasing concentrations of the two drugs on isolated duodenum of rabbits separately. A fixed dose of ranitidine was then selected for studying its potentiating effect on increasing concentrations of metoclopramide on isolated duodenum of rabbits utilizing iWorx Data acquisition unit AHK/214.

Results: Ranitidine enhances the prokinetic effect of metoclopramide.

Conclusion: Ranitidine enhances the contractile effect of the gut in vitro and potentiates the prokinetic effect of metoclopramide. So we conclude that ranitidine is a better choice for patients suffering from gastroesophageal reflux disease (GERD) along with gastroparesis as it enhances the prokinetic effect of metoclopramide.

Keywords: Gastro-intestinal motility, Metoclopramide, Prokinetic, Ranitidine.

INTRODUCTION

Gastroparesis and delayed gastric emptying are two terms often used indistinguishably which has caused considerable perplexity in the clinical settings[1]. In literal terms gastroparesis stands for "paralyzed stomach"[2]. Gastroparesis is a functional disorder with no mechanical obstruction in the upper gastrointestinal tract (GIT)[3].

Females show four times higher predominance of delayed gastric emptying than their male counterparts which can be explained by hormonal changes[4]. Abdominal pain is the predominant symptom in approximately 20 % of the patients with pain being localized mostly to the epigastric region. Gastroparesis stays silent as long as the patient is in a fasting state and triggered by ingestion of food or liquids[5]. Gastroparesis is a heterogeneous disorder and can occur in a number of clinical settings with diabetes as the main underlying etiological factor. Approximately 40% of the cases of gastroesophageal reflux disease (GERD) present with delayed gastric emptying[6]. The underlying cellular defect in gastroparesis are absent expression of neuronal nitric oxide synthase (nNOS) and absent interstitial cells of cajal (ICC). The forefront of pharmacological therapy for patients of gastroparesis are the prokinetic drugs. These drugs facilitate the propulsive movement of food through the gut by increasing the motor activity[7].

GERD is a condition in which the gastric contents are refluxed back into the oesophagus causing symptoms and complications[8].

GERD was considered an insignificant problem until its resurgence almost 10-20 years ago. This condition is difficult to diagnose on clinical grounds alone and requires abetment

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from invasive procedures like endoscopy[9]. Gastro-oesophageal reflux disease (GERD) which occurs in recumbent position requires overnight acid suppression with ranitidine as it is better than the proton pump inhibitors[10].

Gastroparesis is the core perpetrator behind refractory or drug resistant gastroesophageal reflux disease (GERD). An estimated 40% of these pharmacologically drug resistant patients of GERD who eventually seek for anti-reflux surgeries have underlying causal gastroparesis. Anti-reflux surgery can be healing for GERD but not for its core cause that is gastroparesis[11].

Histamine receptor type-2 (H2) antagonists, ranitidine and nizatidine (not cimetidine and famotidine) ameliorate gastroparesis in addition to their well-established role of gastric acid inhibition[12]. Prokinetic activity of H2 blockers is believed to be a consequence of acetylcholinesterase (AchE) inhibition, the enzyme that degrades acetylcholine (Ach) in the synaptic cleft. Inhibition of AchE increases the Ach levels which then increases the motility of the gut[13]. Ranitidine fortunately is a relatively well tolerated drug with very few side effects. The side effects are mild and include diarrhoea and headache[14].

Metoclopramide, a prokinetic known to us all, is an antagonist of the dopamine receptor type-2 (D2) and an agonist at 5-hydroxytryptamine receptor type-4 (5-HT4). Together both these actions lead to an increase in levels of Ach and thus increase the emptying of the stomach[15]. Central nervous system adverse effects of metoclopramide include acute dystonias, akathisia, parkinsonism, tardive dyskinesia, neuroleptic malignant syndrome and rabbit syndrome or perioral tremor[16].

This study was designed to evaluate the potentiation of prokinetic activity of metoclopramide by an H2 blocker ranitidine on isolated duodenum of rabbits. This combination was explored for patients of GERD who have underlying gastroparesis as a root cause. Ranitidine in combination with metoclopramide can then be a potentially viable alternative in place of proton pump inhibitors for these GERD patients.

MATERIAL AND METHODS

This randomised controlled trial was conducted at the department of pharmacology & therapeutics in collaboration with the physiology department, Army Medical College Rawalpindi. Study protocol approval was sought from Ethics Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. Animals (rabbits) locally bred of species Oryctalagus cuniculus both male and female were initially selected through non-probability convenience method and then divided randomly by lottery method into 03 groups. Sample size was of 18 animals. They were higher subdivided randomly into 03 groups, each group having 6 animals. They were allowed to acclimatize to the new environment for 7-8 days at animal house of Army Medical College under standard laboratory

Table: Response and percent response of metoclopramide alone and in combination with ranitidine.

Dose of metoclopramide (µg)	Log Dose	Response (mV) ± SEM of metoclopramide alone	Response (mV) ± SEM of ranitidine + metoclopramide	*p*-value	Percent response of metoclopramide alone	Percent response of ranitidine + metoclopramide
6	0.8	0.074 ± 0.011	0.166 ± 0.014	<0.001**	83	187
12	1.1	0.075 ± 0.008	0.168 ± 0.010	<0.001**	84	189
18	1.3	0.083 ± 0.010	0.169 ± 0.010	<0.001**	90	191
24	1.4	0.086 ± 0.014	0.173 ± 0.013	<0.001**	97	194
30	1.5	0.089 ± 0.017	0.175 ± 0.019	0.012*	100	197

*p**< 0.05 significant
*p***<0.01 highly significant

conditions (12 hour light/dark cycle, 24° C and 50-70% humidity). Commercial standard food (carrots, choker and grains) and tap water was provided ad *libitum*. Tyrode's solution was used for the study as a nutrient solution, the composition of which was NaCl: 137 mM (08.00 g), KCl: 2.7 mM (0.20 g), CaCl2:1.8 mM (0.20 g), MgCl2: 1.05 mM (0.10 g), NaHCO3: 12.0 mM (1.00 g), NaH2PO4: 0.42 mM (0.05 g), Glucose: 5.6 mM (1.00 g) dissolved in 1 L of distilled water. Overnight fasting rabbits were sacrificed and dissected. The duodenum was excised and placed in Tyrode's solution contained in organ bath of 50 ml capacity and bubbled with 100% O_2[17] and maintained at a temperature of 37 ± 2°C[18]. The tissue was allowed a period of equilibrium of 15-30 min during which Tyrode's solution was changed twice. One end of the duodenum was attached to the bottom of the oxygen tube bath and the other was connected by a silk thread to a Research Grade Isometric Force Transducer DT-475 (USA). The isotonic duodenal muscle activity was measured through the displacement transducer[19]. Three groups were made as under: Group 1: Dose response curve was made using cumulatively increasing concentrations of ranitidine (1.4-70 µg) on isolated piece of duodenum (n=6) of rabbits[20]. The isolated piece of duodenum was allowed an initial equilibrium period of 15 min, after which 0.4 ml (1.4 µg) of 10-5 M of ranitidine was added to the organ bath. The isolated duodenal muscle activity was recorded with the aid of DT-475 displacement transducer. Subsequent doses added to the organ bath included 0.6 ml (2.1 µg) and 0.8 ml (2.8 µg). The tissue was then washed with Tyrode's solution twice to relax passively. The next concentration added was 10-4 M and the volumes used were 0.2 ml (7.0 µg), 0.4 ml (14 µg), 0.8 ml (28 µg) and 1 ml (35 µg). The smooth muscle activity was recorded after which the tissue was again washed twice with Tyrode's solution. Then 0.2 ml (70 µg) of 10-3 M concentration of ranitidine. Cumulative dose response curve was constructed by plotting increasing concentrations of ranitidine on x-axis and the percent response on y-axis. The maximal response of ranitidine was taken as 100 percent and then a submaximal dose of ranitidine was selected to be used as a fixed dose for pre-treating group 3 to observe the potentiating effect of ranitidine on metoclopramide. Six groups of experiments were performed in the same way and the mean response for each dose was calculated. Semi log dose response curve was plotted by taking percent response on y-axis and log dose on x-axis.

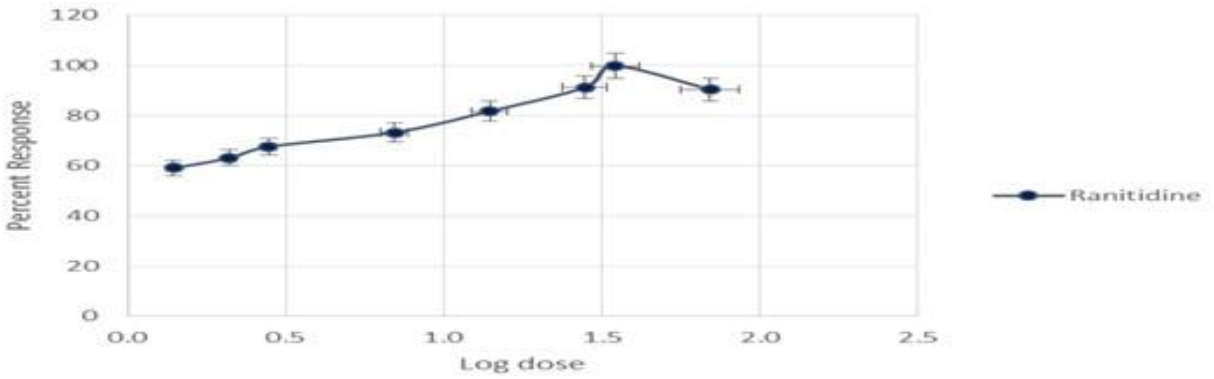

Figure-1: Semi log dose response curve of ranitidine.

Group-2: Dose response curve was made using cumulatively increasing concentrations of metoclopramide (6-30 µg) on isolated piece of duodenum (n=6) of rabbits[21]. After allowing an initial equilibrium period of 15 min to the isolated tissue variable doses of 100 µM of metoclopramide, 0.2 ml (6 µg), 0.4 ml (12 µg), 0.6 ml (18 µg), 0.8 ml (24 µg) and 1.0 ml (30 µg) were

added to the organ bath. The isolated duodenal muscle activity was recorded on iWorx via displacement transducer. The same experiment was performed for six times and the mean responses were calculated. The maximum response was taken as 100 percent and other responses were compared to it. Semi log dose response curve was plotted by taking percent responses on y-axis and log dose on x-axis.

Group-3: Dose response curve was constructed using fixed dose of ranitidine (28 µg) plus cumulatively increasing concentrations of metoclopramide (6-30 µg) on isolated piece of duodenum (n=6) of rabbits[21]. The tissue was allowed an equilibration period of 15 min before adding a fixed dose of ranitidine. A submaximal dose of 0.8 ml 0f 10-4 M (28 µg) of ranitidine was used to pre-treat the tissue. The prokinetic activity of ranitidine was recorded with the help of displacement transducer. Without washing the tissue after addition of ranitidine, increasing concentrations of metoclopramide 0.2 ml (6.0 µg), 0.4 ml (12.0 µg), 0.6ml (18.0 µg), 0.8 ml (24.0 µg) and 1.0 ml (30.0 µg) were added to the organ bath. The same procedure was repeated on six different tissues from six rabbits and the mean was calculated. The maximal response was taken as 100 percent and other responses were compared to it. Semi log-dose response curve was made by plotting percent responses on y-axis and log dose on x-axis.

Statistical analysis

The results have been stated as Means ± Standard Error of Means (SEM). The difference between the two observations (group 2 and 3) was calculated using Independent sample Student's "t" test. The difference among groups 2 and 3 was considered to be significant statistically if $p<0.05$ and highly significant if $p<0.01$.

RESULTS

Ranitidine produced a dose dependent reversible contraction of the isolated duodenum of rabbits (fig-1). A series of six experiments were performed and the mean ± SEM values of responses to increasing concentrations of ranitidine 1.4 µg, 2.1 µg, 2.8 µg, 7.0 µg, 14.0 µg, 28.0 µg, 35.0 µg and 70 µg were 0.086 ± 0.004, 0.092 ± 0.004, 0.100 ± 0.010, 0.111 ± 0.009, 0.124 ± 0.014, 0.136 ± 0.011, 0.123 ± 0.008 mV respectively. The response of ranitidine at 35 µg was considered as 100 percent and other responses when compared with it came out to be 59, 63, 68, 73, 82, 91 and 90 percent respectively. Metoclopramide produced a dose dependent reversible contraction of the isolated duodenum of rabbits. The mean ± SEM values and the percent responses to metoclopramides increasing concentrations are stated in table.

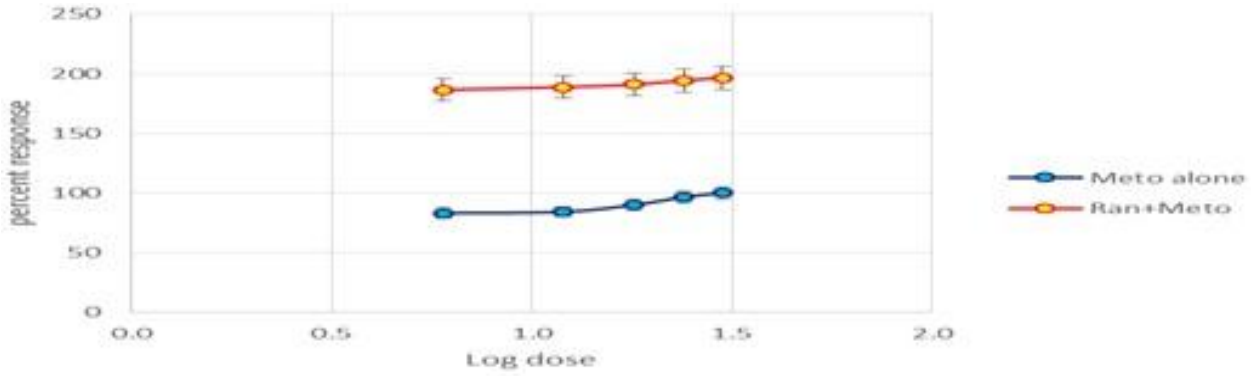

Figure-2: Semi log-dose response curve of metoclopramide alone and ranitidine with metoclopramide.

The mean ± SEM values for the responses when fixed dose of ranitidine (28µg) was added with cumulatively increasing doses of metoclopramide are shownas in the table. The

percent response for each successive dose of metoclopramide was calculated taking the 100 percent response of metoclopramide alone as maximum (0.089 mV) and all the responses of ranitidine + metoclopramide were compared with it and came out to be 187, 189, 191, 194 and 197 percent respectively (table).

The mean ± SEM values and percent responses of groups 2 and 3 when compared were found to be statistically highly significant (mean *p* value <0.001**).

The log dose response curve of ranitidine + metoclopramide when plotted with metoclopramide alone was shifted to the left and upwards. The percent responses of metoclopramide alone was 91 percent and was 191 percent when pre-treated with ranitidine and the results were highly statistically significant.

DISCUSSION

The research proposal which led to the formulation of this project was conceived amongst reports of emerging prokinetic role of a well-known H blocker, ranitidine, which was primarily being used for the treatment of peptic ulcers. In the first group of experiments, the prokinetic potential of ranitidine was explored by adding cumulatively increasing concentrations on isolated duodenum of rabbits. Ranitidine was able to produce a marked increase in the amplitude of contractions of isolated duodenum. Kusano and his co-researchers proposed that ranitidine causes increased cholinergic transmission[22]. Zai and his colleagues explained that ranitidine increases the motility of the gastrointestinal tract by increasing the levels of acetylcholine either by direct cholinergic agonism or indirectly either by increasing the release of acetylcholine from cholinergic nerves or by acetylcholinesterase inhibition[23]. Metoclopramide dose dependently enhanced the motility of duodenum when added at a cumulatively increasing dosage of 6-30 µg and 0.136 mV was recorded as the maximum effect at 35 µg dose.

Metoclopramide augments the motility of the gut by its peripheral anti-dopaminergic action[24]. Dopamine has an inhibitory influence on the levels of acetylcholine in the smooth muscle of the gastrointestinal tract and by removing this inhibition as well as by causing increased sensitivity of the muscarinic receptors, metoclopramide augments the motility of the gut[25]. The dose response curve of metoclopramide when added with ranitidine was shifted to the left when compared to metoclopramide alone group and the result was found to be statistically significant. Metoclopramide increases gut motility by its anti-dopaminergic and 5-HT4 agonist activity[26] whereas ranitidine acts as a prokinetic either by direct cholinergic agonism or by indirect acetylcholinesterase inhibition[23]. So a diversity in the mechanism of action might be responsible for the increased contractile response when the two are used in combination.

CONCLUSION

Ranitidine enhances the contractile effect of the gut in vitro and potentiates the prokinetic effect of metoclopramide. So we conclude that ranitidine is a better choice for patients suffering from gastroesophageal reflux disease (GERD) along with gastroparesis as it enhances the prokinetic effect of metoclopramide.

CONFLICTS OF INTEREST

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

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