

ORIGINAL ARTICLE

RANITIDINE CAN POTENTIATE THE PROKINETIC EFFECT OF ITOPRIDE AT LOW DOSES- AN IN VITRO STUDY

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Background: Gastroparesis and GERD occur concomitantly in 40 percent of the cases. Prokinetic drugs and acid blockers are employed as the main treatment modality. Ranitidine is an acid blocker with additional prokinetic activity and Itopride is a known prokinetic drug. This study was designed to observe the synergistic potentiating prokinetic effect of Ranitidine on itopride on isolated duodenum of rabbits. **Methods:** Ranitidine (10^{-5} - 10^{-3}) and itopride (10^{-6} - 10^{-5}) were added in increasing concentrations to isolated duodenum of rabbits and contractions were recorded on PowerLab Data acquisition unit AHK/214. Cumulative dose response curves were constructed. The potentiating prokinetic effect of Ranitidine on itopride was seen by using a fixed dose of ranitidine and cumulatively enhancing doses of itopride on iWorx. **Results:** Ranitidine and itopride produced a dose dependent reversible contraction of the isolated tissue of rabbits with ranitidine showing a max response of 0.124mV and itopride showing a maximum response of 0.131mV. Ranitidine was able to potentiate the prokinetic effect of itopride at low doses but at high dose the effect began to wane off. **Conclusion:** Ranitidine and itopride produce a statistically significant synergistic potentiating prokinetic effect at low doses in vitro.

Keywords: Ranitidine; Itopride, Prokinetic, Outcome

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INTRODUCTION

Drug resistance is a major setback in the medical treatment of gastroesophageal reflux disease (GERD). Gastroparesis is found to be the main cause behind drug resistant GERD in 40 percent of the cases. Anti-reflux surgery can be curative for GERD but not for gastroparesis.¹ Prokinetic drugs are employed for the medical treatment of gastroparesis. These drugs facilitate gastokinetic movement of the gut by increasing the motor activity.² Traditionally drugs which increase the cholinergic stimulation, either directly or indirectly are the mainstay of the remedial measures. Some Dopamine receptor type-2 (D_2) blockers increase GI motility by inhibiting the negative influence on cholinergic nerve terminals in the gut. Acid blocking drugs like ranitidine are used along with pro-kinetics in such disorders. Gastroesophageal reflux disease (GERD) requires overnight acid suppression with ranitidine as it is better than the proton pump inhibitors.³

Itopride has a dual action of inhibiting AchE enzyme and antagonizing D_2 receptors.⁴ Both animal and human studies confirm the ability of itopride to increase the gastric emptying.⁵ The outcome of inhibiting AchE and antagonizing D_2 receptors is an increase in the levels of the Ach which accelerates the motility of the gut and enhances the emptying of food from the stomach.⁶ Itopride also has anti-emetic properties.⁵ As itopride antagonizes D_2 receptors, so it is also being used in inhibiting apomorphine

induced vomiting at the same doses as required to stimulate gastric motility.⁷

Itopride is a highly polar prokinetic drug the inability of which to cross the blood brain barrier (BBB) makes it an attractive substitute to metoclopramide in the management of a patient with gastroparesis.⁸ In humans itopride is metabolized to N-oxide when tertiary amine undergoes oxidation by Flavin monooxygenase (FMO).⁹ Itopride is used for the relief of symptoms of functional dyspepsia and those resulting from reduced gastrointestinal motility.¹⁰

Itopride, unlike metoclopramide, does not cause Central Nervous system (CNS) related adverse effects neither does it cause prolongation of QT interval or elevate the levels of prolactin.⁸ The side effects ascribed to itopride include the gastrointestinal system effects like diarrhoea and pain in the abdomen.¹¹

Ranitidine acts at the histamine type-2 (H_2) receptors located on parietal cells in the stomach and antagonizes histamine-stimulated acid release.¹² In some recent studies the prokinetic role of two of the H_2 antagonists, ranitidine and nizatidine (not cimetidine and famotidine) has been claimed to be better than a lot of well-known prokinetic drugs and thus they are an attractive option in gastroparesis.¹³ Prokinetic activity of H_2 blockers is the result of either indirect AchE inhibition thus increasing the levels of Ach or by direct cholinergic action on the gastric smooth muscle.¹⁴

Ranitidine is absorbed well upon oral intake with bioavailability ranging widely on a large scale between individuals. The pharmacokinetics of the oral and the intravenous preparations do not differ markedly. The plasma protein binding is about 23 percent. Its distribution occurs in total body water (TBW) because of its hydrophilic nature.¹⁵ The major organ for the metabolism of the drug is the liver while kidney is the main route for its excretion with 70 percent of it being excreted unchanged.¹⁶ Ranitidine has largely replaced cimetidine because of ease of dosing, improved safety profile and less potential for drug-drug interaction.¹⁷

This study was designed to observe the enhancement of prokinetic action when the two strong prokinetics, Ranitidine and Itopride are used together on isolated tissue of rabbits.

MATERIAL AND METHODS

This randomised controlled trial (Experimental study) was conducted in the Department of Pharmacology & Therapeutics, Army Medical College Rawalpindi. Ethics Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi approved the study protocol. Animals (rabbits) both male and female, locally bred of species *Oryctolagus cuniculus* were initially selected through non-probability convenience method and then later divided randomly by lottery method into 03 groups. Sample size was of 18 animals, each group having 6 animals. 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 % O₂¹⁸ and maintained at a temperature of 37±2 °C.¹⁹ 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.²⁰ 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 rabbit. The isolated piece of duodenum was allowed an initial equilibrium period of 15 min, after which 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

2.1 µg and 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 doses used were 7.0 µg, 14 µg, 28 µg and 35 µg. The smooth muscle activity was recorded after which the tissue was again washed twice with Tyrode's solution. Then 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.

Group 2: After the isolation of tissue it was placed in the tyrodes solution for 15 min for equilibration. Then itopride was added in increasing doses of 80 ng, 160 ng, 240 ng, 320 ng and 400 ng of the same concentration. The tissue was washed with tyrode's solution. The isolated smooth muscle activity of the duodenum was recorded on PowerLab. The experiment was done on six different animals and the mean response was calculated. Percent responses were calculated and the maximal response was taken as 100 percent. Responses of individual concentrations were compared to it. Semi log-dose response curve was made by plotting percent responses on the y-axis and log dose on x-axis.

Group 3: The isolated piece of duodenum was equilibrated in tyrodes solution for 15 min after which 28 µg of ranitidine was added. The motility enhancing activity of ranitidine on the duodenal piece was recorded with DT-475 Displacement Transducer on Labscribe. After the prokinetic effect of ranitidine reached its peak, cumulatively increasing concentrations of itopride were added to the organ bath. Doses of itopride added were 80 ng, 160 ng, 240 ng, 320 ng and 400 ng. The tissue was then washed with tyrodes and then the next concentration of itopride was added. The above experiment was repeated six times and the mean response was calculated for each experiment. Maximal response was taken as 100 percent and other responses were compared to it. Semi log-dose response curve was made by taking percent responses on y-axis and log dose on x-axis.

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 $p < 0.05$.

RESULTS

Ranitidine produced a dose dependent reversible contraction of the isolated duodenum of rabbits. 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. Semi Log dose response curve was plotted by taking log dose on x-axis and percent response on y-axis (Figure-1). Itopride produced a dose dependent reversible contraction of the isolated duodenum of rabbits. A series of six experiments were performed and the mean±SEM of responses to varying doses of itopride 80 ng, 160 ng, 240 ng, 320 ng and 400 ng were 0.110±0.011 mV, 0.126±0.011 mV, 0.129±0.010 mV, 0.130±0.009 mV and 0.131±0.006 mV respectively. Percent responses were calculated for all the above-mentioned doses of itopride taking the response of 0.131mV as 100 percent. The percent responses to other concentrations were 84, 88, 94 and 99 percent respectively (Table-1). Semi log-dose response curve was made by plotting percent response on y-axis and log dose on x-axis (Figure-2).

The potentiating prokinetic effect of ranitidine on itopride was recorded on iWorx by adding a sub maximal fixed dose of ranitidine (28 µg) and cumulatively increasing concentrations of itopride on isolated duodenum of rabbits. Cumulative dose response curve was plotted using increasing concentrations of itopride (80.0 ng, 160.0 ng, 240.0 ng, 320.0 ng and 400.0 ng). Each new concentration was added after the achievement of maximal response from the previous concentration. Six experiments were performed and the mean±S.E.M. values of responses to above mentioned doses were 0.137±0.002 mV, 0.111±0.001 mV, 0.112±0.0002

mV, 0.101±0.0001 mV and 0.097±0.0001 mV. Percent responses were calculated for all the above five mentioned doses and the response of itopride alone was taken as 100 percent and the response to other doses were compared with it: 113, 92, 85, 83 and 80 percent respectively. The percentage enhancement at the lowest dose was calculated to be 24 percent which is statistically significant (Table-1). Semi log-dose response curve was made by plotting percent responses on y-axis and log dose on x-axis (Figure-2). The mean *p* value was calculated to be 0.04 which is considered to be statistically significant.

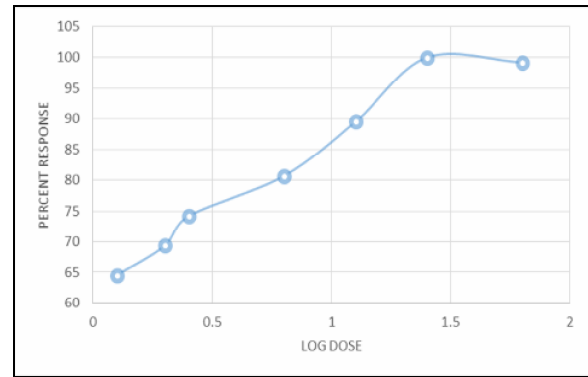


Figure-1: Log Dose Response curve of Ranitidine on isolated duodenum of rabbits (n=6).

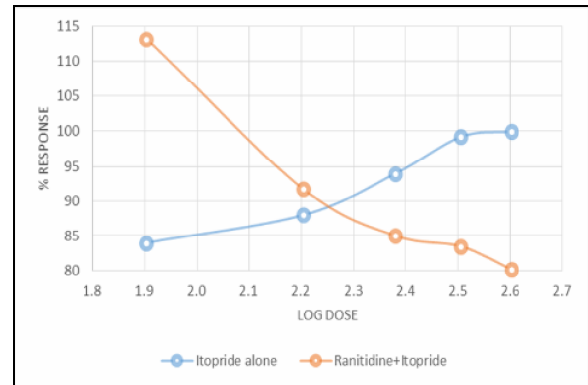


Figure-2: Semi log dose response curve of Itopride alone (blue) compared with ranitidine + itopride (red). Log dose is plotted on x-axis and percent response on y-axis.

Table-1: Mean and percent responses of itopride alone compared with the mean and percent responses of itopride and ranitidine.

Dose of Itopride (ng)	Log dose	Mean Response of Itopride (mV+SEM) n=6	Mean Response of Ranitidine+Itopride (mV+SEM) n=6	Percent Response of Itopride alone (%)	Percent Response of Ranitidine+Itopride (%)
80.0	1.9	0.110 ± 0.011	0.137±0.002	84	113
160.0	2.2	0.126±0.011	0.111±0.001	88	92
240.0	2.4	0.129±0.010	0.112±0.0002	94	85
320.0	2.5	0.130±0.009	0.101±0.001	99	83
400.0	2.6	0.131±0.006	0.097±0.001	100	80

DISCUSSION

Ranitidine was able to produce a marked increase in the amplitude of contractions in the first group of experiments. The maximum effect was recorded at 35 µg and was considered as 100 percent and the responses to doses of 1.4 µg, 2.1 µg, 2.8 µg, 7.0 µg, 14.0 µg, 28.0 µg and 70 µg when compared with it came out to be 59, 63, 68, 73, 82, 91 and 90 percent respectively. The submaximal dose of ranitidine of 28 µg was chosen as the fixed dose to be used to observe the potentiating prokinetic effect on itopride in vitro. Kusano and his co-researchers proposed that ranitidine causes increased cholinergic transmission.²¹ Zai and his fellows explained that ranitidine enhances the motility of the gastrointestinal tract either by direct cholinergic agonism or indirectly either by increasing the release of acetylcholine from cholinergic nerves or by inhibition of acetylcholinesterase enzyme thereby ultimately increasing the levels of acetylcholine.²²

Itopride was added in a cumulatively increasing concentration of 10^{-6} - 10^{-5} M and showed statistically significant increase of motility in isolated duodenum. The maximum effect recorded was 0.131 mV and was taken as 100 percent and the responses to other doses were compared to it. The response of itopride was 57 percent when compared to the 100 percent response of ranitidine. Our result was found to be in conformance with the results of Iwanaga and his co-workers who concluded that itopride is a gastroprokinetic agent of the upper gastrointestinal tract and increases the contraction of the gastric antrum as well as the duodenum by blocking D₂ receptors and inhibiting AchE enzyme, both mechanisms leading to an increase in levels of Ach.²³ Lim and his colleagues performed a similar study in 2008 and concluded that itopride has a stimulatory action on the gut by increasing Ach levels on M₃ receptors.²⁴

The third group of experiments was conducted to observe the synergism of ranitidine with itopride, two very strong prokinetics. Ranitidine was added in a fixed dose of 28 µg and itopride added in a cumulatively increasing concentration of 80–400 ng. The maximum effect was 0.137 mV at the lowest dose of 80 ng. The responses were found to be statistically significant. Initially ranitidine was able to enhance the prokinetic action of itopride at lowest prokinetic dose but then with increasing dose of 140 ng the effect began to wane off and came out to be 0.111 mV. The response at the highest dose of 400 ng was 0.097 mV. The combined prokinetic effect of the two agents came out to be less than that of itopride alone. This might be explained by the saturation of inhibition of AchE enzyme, mechanism which is

common to the prokinetic action of both the drugs. At the lower dose, the enhancement of prokinetic action caused by ranitidine and itopride was a statistically significant (0.02) 24 percent. Iwanaga and his fellow researchers explained the nature of nature of inhibition of Ach enzyme by itopride and ranitidine. Itopride inhibits the AchE enzyme non-competitively only whereas ranitidine inhibits AchE enzyme both competitively and non-competitively.²⁵

CONCLUSION

Itopride and ranitidine enhance the prokinetic action when used together at low prokinetic doses but with the increase of dosage, the combined prokinetic effect becomes less than the individual prokinetic dose. This waning effect at high doses may be explained by prokinetic mechanism of action which is common to both the drugs, that is acetylcholinesterase inhibition.

AUTHORS' CONTRIBUTION

AIB: Conception of idea, drafting of article, collection of data. BTK: Revision of article. AK & QUZK: Proof reading

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