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SYNERGISTIC DRUG-DRUG INTERACTION;
RANITIDINE AND LEVOSULPIRIDE-IN VITRO

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ABSTRACT: Introduction: Ranitidine is known to us all as an anti-ulcer drug which acts by blocking H_2 receptors in the stomach parietal cells. However its role in this category has been understated. We studied its prokinetic effect on isolated duodenum of rabbits and its synergistic interaction with Levosulpiride. The purpose of the study was to see if the two drugs do have a prokinetic effect and whether the combined effect is greater than the individual drugs. **Study Design:** Laboratory based Randomised controlled trial. **Period:** November 2014 to November 2015. **Setting:** The study was carried out in the multidisciplinary laboratory at Army Medical College after approval from Animals ethics committee. **Material and methods:** Dose response curve was constructed using cumulatively increasing concentrations of Ranitidine (Group 1) and Levosulpiride (Group 2). The synergistic prokinetic drug-drug interaction of Ranitidine and Levosulpiride was observed in Group 3 on iWorx Data acquisition unit (PowerLab). **Results and Conclusion**: Ranitidine produced a dose dependent reversible contraction of the isolated duodenum and the maximum effect was recorded at 35 μg as 0.136 mV. Levosulpiride produced a maximum contraction of 0.088 mV at 70 μg. Ranitidine and levosulpiride curve was shifted to the left and upwards of levosulpiride alone. The percent responses of levosulpiride alone was 90 percent and with ranitidine was 122 percent. Ranitidine and levosulpiride have a synergistic prokinetic interaction in vitro. **Conclusion:** Ranitidine and levosulpiride have a synergistic prokinetic drug-drug interaction in vitro.

Key words: Ranitidine, levosulpiride, prokinetic.

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INTRODUCTION

Ranitidine enhances the gut motility by reversibly inhibiting the enzyme which degrades acetylcholine, that is Acetylcholinesterase (AchE) and thus increasing the motility. The prokinetic effect has been postulated to be better than some of the well-known prokinetic agents.[1] Its prokinetic activity has been proposed to be a result of Acetylcholinesterase enzyme (AchE) inhibition.[2]

Levosulpiride, a benzamide derivative is the levorotatory arrangement of sulpiride.[3] Levosulpiride in addition to the D_2 antagonist action has a slight agonist activity at 5-HT_4 receptors and antagonist at 5-HT_3 receptor and is reported to be better than cisapride in improving symptoms like nausea, vomiting and early satiety.[4] Agonist activity at 5-HT_4 receptors is suggested to be the main mechanism leading to increase in the kinetic activity of the gut.[5] Levosulpiride increases the pressure in the sphincter of lower oesophagus, increases emptying from the stomach and also decreases the gastric sensation by raising the stomach threshold to distention. Levosulpiride thus improves the general well-being of the patient by decreasing the impact of the symptoms on patients daily routine.[6]

Levosulpiride has anti-psychotic, anti-depressive and anti-ulcer effects. Hence used for the treatment of schizophrenia, depressive disorders, peptic ulcers whether gastric or duodenal and irritable colon.[7]

This study has been designed to observe the

synergistic prokinetic drug-drug interaction of Ranitidine and levosulpiride.

METHODOLOGY

This study was a laboratory based Randomised controlled trial conducted at the department of Pharmacology and Therapeutics in collaboration with the Centre for Research in Experimental and Applied Medicine at the Army Medical College Rawalpindi, Pakistan. The institutional Animals Ethics Committee approved of this study. The study was completed in one year. Eighteen Healthy locally bred Rabbits, both male and female were procured from the market and divided into three groups, each group consisting of six animals. Animals were selected through non probability convenience method and later divided by lottery method. Overnight fasting rabbits were sacrificed, dissected and the small intestine removed. Duodenum was isolated, washed with normal saline, cut into one inch pieces, placed in Tyrode's solution contained in organ bath of 50 ml capacity, bubbled with 100 percent O_2[8] and maintained at a temperature of 37 ± 2ºC.[9] 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). Power-lab was used to record the isolated duodenal contractions.

Group 1

The isolated piece of duodenum was allowed an initial equilibrium period of 15 min after which Ranitidine was added in cumulatively increasing doses of 2.1 µg, 2.8 µg, 7.0 µg, 14.0 µg, 28.0 µg, 35.0 µg, and 70.0 µg to the organ bath and isolated duodenal muscle activity was recorded on Powerlab. The Maximum response was taken as 100 percent and responses to other doses were compared with it. A Semi log dose response curve was plotted with log dose on x axis and Percent response on y axis. A Submaximal dose of ranitidine was selected to pretreat the tissue in group 3.

Group 2

The isolated duodenum of the rabbit was equilibrated in Tyrode's solution for 15 min and then cumulatively increasing concentrations of levosulpiride (1.4 µg, 2.1 µg, 7.0 µg, 14.0 µg, 21.0 µg, 70.0 µg and 210.0 µg) were added to the organ bath. The duodenal smooth muscle activity was recorded by using a Displacement Transducer on iWorx. The maximum respose was taken as 100 percent and the responses to other doses were compared with it. Dose Response curve was plotted using log dose on x axis and percent response on y axis.

Group 3

Dose Response Curve was plotted using a submaximal fixed dose of Ranitidine (28 µg) and cumulatively increasing concentrations of Levosulpiride (1.4 µg, 2.1 µg, 7.0 µg, 14.0 µg, 21.0 µg, 70.0 µg and 210.0 µg) and compared with the dose response curve of levosulpiride alone to observe the potentiating effect of Ranitidine.

After the plateau phase of ranitidine was reached, gradually increasing concentrations of levosulpiride were added to the organ bath.

Statistical analysis

The results have been stated as Means ± Standard Error of Means (SEM). The arithmetic means of responses of contractile activity of isolated duodenum were calculated. The percent responses and percentage enhancement were calculated using the Microsoft Office Excel 2013 and the values were considered significant if p was less than 0.05. The difference between the two observations was calculated using Independent Sample Student's "t" test. The difference was established to be statistically significant if the value of p was less than 0.05.

RESULTS

Response to Ranitidine

Ranitidine produced a dose dependent reversible contraction of the isolated duodenum of rabbits. Dose response curve was plotted using cumulatively increasing doses of ranitidine (2.1 µg, 2.8 µg, 7.0 µg, 14.0 µg, 28.0 µg, 35 µg and 70 µg) and the mean ± SEM were 0.086 ± 0.004 mV, 0.092 ± 0.004 mV, 0.100 ± 0.010 mV, 0.111 ±

0.009 mV, 0.124 ± 0.014 mV, 0.136 ± 0.011 and 0.123 ± 0.008 mV respectively. The maximum response was obtained at 35 μg and was taken as 100 percent and the responses at other doses were 63, 68, 73, 82, 91 and 90 percent respectively.

Response to Levosulpiride

Levosulpiride produced a dose dependent reversible contraction of the isolated duodenum of rabbits. Cumulative dose response curve was plotted using increasing concentrations of levosulpiride. The doses of which are 1.4 μg, 2.1 μg, 7.0 μg, 14.0 μg, 21.0 μg, 70.0 μg and 210 μg and the mean ± SEM of responses to concentrations of ranitidine were 0.068 ± 0.016 mV, 0.070 ± 0.013 mV, 0.078 ± 0.008 mV, 0.082 ± 0.008 mV, 0.085 ± 0.010 mV, 0.088 ± 0.009 mV, 0.086 ± 0.012 mV respectively. Percent responses were calculated for all the above mentioned doses of levosulpiride taking the response of 0.088 mV as 100 percent. The percent responses to other concentrations were 77, 79, 88, 93, 96, 97 percent respectively. Semi log dose response curve was plotting by taking percent response on y-axis and log dose on x-axis (Figure-1).

The potentiating prokinetic effect of ranitidine on levosulpiride was recorded on iWorx by adding fixed dose of ranitidine and cumulatively increasing concentrations of levosulpiride on isolated duodenum of rabbit.

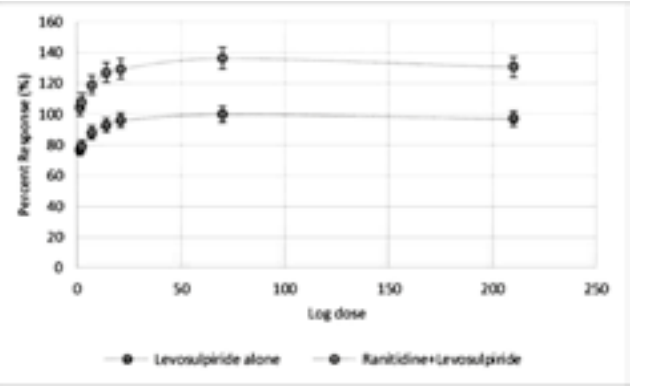

Figure-1. Dose response curve of Levosulpiride alone (blue) and levosulpiride with ranitidine (orange)

Cumulative dose response curve was plotted using increasing concentrations of levosulpiride. The doses for the above mentioned 1.4 μg, 2.1 μg, 7.0 μg, 14.0 μg, 21.0 μg, 70.0 μg and 210 μg. Each new concentration was added after the achievement of maximal response from the previous concentration. Six experiments were performed and the mean ± SEM of responses to above mentioned doses were 0.092 ± 0.008 mV, 0.095 ± 0.009 mV, 0.105 ± 0.002 mV, 0.112 ± 0.007 mV, 0.114 ± 0.010 mV, 0.120 ± 0.006 mV, 0.115 ± 0.002 mV. Percent responses were calculated taking the 100 percent response of levosulpiride alone (0.088mV) as maximum and the responses of Ranitidine + levosulpiride were compared with it and came out to be; 105, 108, 119, 127, 130, 136 and 131 percent respectively (Table-I).

Dose of Levosulpiride (μg)	Response of Levosulpiride alone (mV)	Response of Ranitidine +levosulpiride (mV)	P value	Percent Response of Levosulpiride alone	Percent Response of Ranitidine +levosulpiride	Percentage enhancement (%)
1.4	0.068 ± 0.016	0.092 ± 0.008	0.1081	77	105	26
2.1	0.07 ± 0.013	0.095 ± 0.009	0.0701	79	108	26
7.0	0.078 ± 0.008	0.105 ± 0.002	0.0145*	88	119	26
14.0	0.082 ± 0.008	0.112 ± 0.007	0.0134*	93	127	27
21.0	0.085 ± 0.010	0.114 ± 0.010	0.0420*	96	130	25
70.0	0.088 ± 0.009	0.120 ± 0.006	0.0050**	100	136	27
210.0	0.086 ± 0.012	0.115 ± 0.002	0.0283*	97	131	25

Table-I. Response and Percent response of Levosulpiride alone and in combination with Ranitidine
Mean p value = 0.04
p* < 0.05 Significant
p < 0.05 Highly significant**

The mean ± SEM values of groups 2 and 3 for the doses 1.4 µg, 2.1 µg, 7.0 µg, 14.0 µg, 21.0 µg, 70.0µg and 210 µg when compared were found to be statistically significant (mean p = 0.04). Ranitidine and levosulpiride curve was shifted to the left and upwards of levosulpiride alone. The percent responses of levosulpiride alone was 90 percent and with ranitidine was 122 percent (Figure-2).

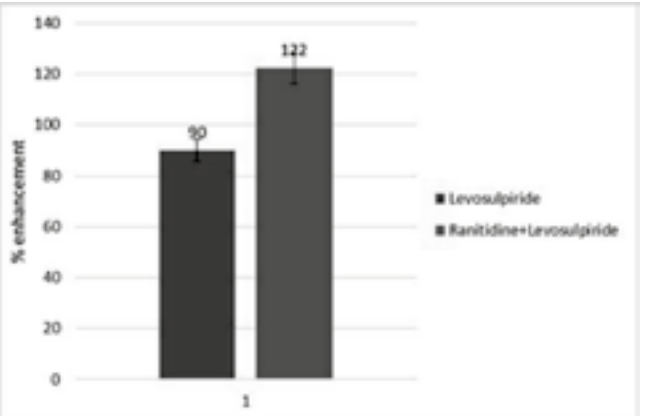

Figure-2. Bar chart showing percent response of Levosulpiride alone (blue) and levosulpiride with ranitidine (orange).

DISCUSSION

This study was designed based on the proposal that Ranitidine, primarily an anti-ulcer and levosulpiride, an anti-psychotic drug both have prokinetic activity. We studied the synergistic drug-drug interaction of ranitidne and levosulpiride. In the first group, the prokinetic potential of ranitidine was studied. 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.[10] 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.[11]

Next we studied the gastroprokinetic effect of levosulpiride in vitro. Levosulpiride produced an increase in the contractile effect of isolated duodenal tissue when added at an increasing concentration. The maximum response was observed at 70 µg and was recoded as 0.088 mV on iWorx/214. Levosulpiride was selected as a prokinetic drug in this study because it has an additional central mechanism of action of relieving depression and anxiety which is a reason for it being prescribed as a common promotility drug.[6]

The last group was conducted to observe the combined contractile effect of ranitidine and levosulpiride on isolated duodenum of rabbits. Levosulpiride when added alone produced a maximum effect of 0.088 mV at 70 µg but when pre-treated with a fixed dose of ranitidine the effect was increased to 0.120 mV. Ranitidine thus was able to enhance the contraction caused by levosulpiride and the percentage enhancement was 35 percent relative to a 100 percent of ranitidine. Dose response curve of ranitidine and levosulpiride was shifted to the left of levosulpiride alone. The percent response of levosulpiride alone was 90 percent and was increased to 122 percent when combined with ranitidine. The means of the responses and percent responses when compared between levosulpiride alone group and levosulpiride pre-treated with ranitidine group were found to be statistically significant at $p < 0.05$.

CONCLUSION

Ranitidine and levosulpiride have a synergistic prokinetic drug-drug interaction in vitro.

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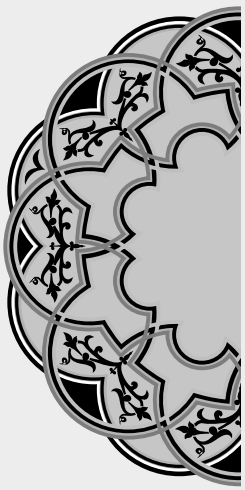

"Do not throw the arrow which will return against you."

Kurdish Proverb

AUTHORSHIP AND CONTRIBUTION DECLARATION

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