ORIGINAL ARTICLE

Potentiation Effects of Acetylcholine at Neuromuscular Junction by Ranitidine

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ABSTRACT

Objective: To evaluate the activity of Ranitidine at neuromuscular junction with and without Pancuronium.

Study Design: Experimental randomized control study.

Place and Duration of Study: Department of Pharmacology, Islamic International Medical College, RIU Rawalpindi from October 2018 to September 2019.

Materials and Methods: Changes in the length (contraction) of rectus abdomininis muscle of frog were recorded using students oscillograph and cumulative dose response curve with Acetylcholine was obtained (control group). The effect of Ranitidine before and after adding Pancuronium was observed using three groups. Statistical analysis of variance (ANOVA) between the groups was performed by using the student's '*t*' test and a P-value < 0.05 was considered statistically significant.

Results: Ranitidine in a dose of 1mM concentration produced a shift of the curve to the left with mean deviation of 61.5% (SEM ± 20.5) showing an enhancement of effects of Acetylcholine. Ranitidine also produced a shift of the curve to the left in the presence of 1µg Pancuronium with the mean deviation of 104.5% (SEM ± 39.7). The shift was statistically significant (P < 0.05) showing the antagonistic effect of Ranitidine on neuromuscular junction (NMJ) blockers like Pancuronium at this concentration.

Conclusion: Ranitidine in a concentration of 1mM increases the effects of Acetylcholine at neuromuscular junction (NMJ) and antagonizes the effects of NMJ blockers like Pancuronium at this concentration.

Key Words: *Acetylcholine, Neuromuscular Junction, Pancuronium, Ranitidine.*

Introduction

Histamine H_2 receptor blockers are widely used in medicine for the treatment of acid peptic disease as they reduce the basal and stimulated acid secretion.[1] They are also used pre-operatively before general anaesthesia for prophylaxis of gastric acid aspiration.[2]

Ranitidine is a competitive antagonist at H_2-receptors.[3] Ranitidine is therapeutically used in the treatment of duodenal ulcer, benign gastric ulcer, stress ulcer, Zollinger Ellison Syndrome, reflux oesophagitis, and other conditions where gastric acid reduction is beneficial.[4] Also used for prophylaxis of gastric acid aspiration during anaesthesia.[5,6]

In the past there is conflicting evidence regarding the interaction between H_2receptor blockers and various neuromuscular junction blockers.

There is evidence that ranitidine can inhibit acetylcholinesterase in low dose and at high doses produce neuromuscular blockade.[7] Also in a study Ranitidine potentiated the effects of neuro muscular junction blocker.[8]

This is of particular interest to anesthesiologists as non-depolarizing neuromuscular junction blockers like Pancuronium are administered during various surgical procedures for muscle relaxation.[9,10] A potential for synergism / potentiation with neuromuscular junction (NMJ) blockers exist in such situations. Thus, there may be a chance of side effects during surgery. This would be clinically relevant because of respiratory depression and prolonged apnoea.

It was therefore pertinent to determine the activity of Ranitidine at neuromuscular junction and its interaction with neuromuscular junction blocker like Pancuronium.

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So, the present study was performed to evaluate the activity of Ranitidine at neuromuscular junction with and without Pancuronium.

Materials and Methods

The experimental randomized control study was carried out in the department of Pharmacology and Therapeutics, Islamic International Medical College, Rawalpindi from October 2018 to September 2019 in collaboration with department of Pharmacology and Therapeutics, Rawalpindi Medical University Rawalpindi. Accredited Ethical Review Committee of Islamic International Medical College approved the research proposal before starting the study. A total of 24 frogs selected randomly, six rectus abdominis muscles in each group were used.[11] Both male and female adult frogs (*Rana-tigrina*).[12] weighing 100 to 150 gm[13,14] were included in the study. Frogs weighing less than 100gm and more than 150 gm were excluded.

The drugs used were Acetylcholine solutions of 10^{-3}, 10^{-4} and 10^{-5} strength, 1g of Pancuronium Bromide (Molecular weight 732.7),[4] 1mM concentration of Ranitidine (Molecular weight 350.9),[15] Frog Ringer solution with the following composition.[16]

NaCl 6.493 g/L, KCl 142mg/L, $CaCl_2$ 133mg/L, NaH_2PO_4 7.68mg/L, $NaHCO_3$ 197mg/L, Dextrose 198mg/L.

Six rectus abdominis muscles from randomly selected frogs were used for recording the observations in each group. Each frog was dissected. The two rectus abdominis muscles were cut across just above the sternum, dissected and shifted to a dish containing frog ringer at room temperature.[17] One muscle was mounted in the organ bath aerated with oxygen and connected to the oscillograph.[18,19] The effects of different drugs were recorded as changes in the muscle length (contraction) via isotonic transducer according to the following schedule.

In Group-I (Control), 1 g Acetylcholine was added to the organ bath and the contraction of the frog's rectus abdominis muscle was recorded on the graph paper for 3 minutes. Next reading with 2g of Acetylcholine was obtained and similarly a cumulative dose response relationship was observed and recorded by doubling the dose of Acetylcholine after every 3 minutes till the maximum or ceiling effect was obtained. Oscillograph was stopped and the Ringer solution was drained and replaced. The tissue was given a rest for 30 minutes and 2nd cumulative dose response curve was obtained on the same rectus abdominis muscle in a similar manner.

Muscles of six different animals were used for the recording as mentioned above. This group was used as a control group for the study.

In Group-II (Pancuronium), cumulative dose response curve was obtained on each of the six preparations with Acetylcholine as already described above. The tissues were given a rest for 30 minutes and a fixed dose of 1 g of Pancuronium was added to the organ bath. After a reaction time of 15 minutes another cumulative dose response curve with Acetylcholine was recorded in the presence of Pancuronium for every preparation.

In Group-III (Ranitidine), cumulative dose response curve was obtained on each of the six preparations with Acetylcholine as already described above. The tissues were given a rest for 30 minutes and a fixed dose of 1 mM solution of Ranitidine was added to the organ bath. After a reaction time of 15 minutes another cumulative dose response curve with Acetylcholine was recorded in the presence of Ranitidine for every preparation.

In Group-IV (Pancuronium + Ranitidine), cumulative dose response curve was obtained on each of the six preparations with Acetylcholine as already described above. The tissues were given a rest for 30 minutes and a fixed dose of 1 g Pancuronium and 1 mM Ranitidine were added to the organ bath. After a reaction time of 15 minutes another cumulative dose response curve with Acetylcholine was recorded in the presence of Pancuronium and Ranitidine for every preparation.

Statistical analysis of variance (ANOVA) between the groups was performed by using the student's 't' test and a P-value < 0.05 was considered statistically significant.

Results

Observations of Groups

Group I (Control Group)

In a series of six experiments the mean ± SEM values of responses for 1, 2, 4, 8, 16, and 32 µg of Acetylcholine were recorded. Percent responses were calculated by taking the response with 32 µg as 100% and were 12%, 31%, 57%, 82%, 95%, and 100% for the above-mentioned doses respectively. After

washing the preparations with frog-Ringer solution and after an interval of 30 minutes the percent responses were 8, 24, 50, 80, 92 and 96 % for each dose respectively. Semi log dose response curves were plotted by taking the percentage responses which showed that the second curve i.e., after washing with frog-Ringer solution and rest on an average shifted to the right and downwards. The deviation was slight which started with the 1st dose of 1 µg and continued in the entire extent of the curve. Percent deviation was calculated for each dose and was 29.4 %, 23.9%, 11.9%, 2.5%, 2.9%, and 4.1% respectively, with mean 12.45%. (SEM± 4.77). P > 0.05.

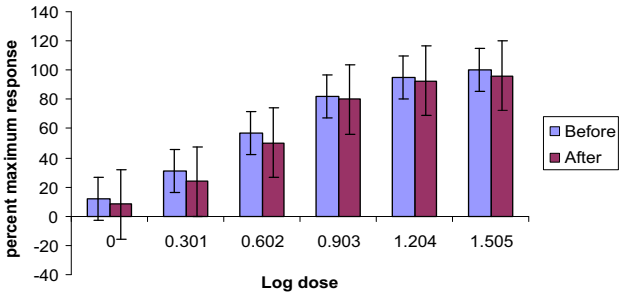

Fig 1: Semi Log Dose Percent Response Bars for Acetylcholine Induced Contractions

Group II (Pancuronium)

In a series of six experiments the mean ± SEM values of responses for 1, 2, 4, 8, 16, 32, 64, and 128 µg, of Acetylcholine were recorded. Percent responses were calculated by taking the response with 128 µg as 100% and were 6%, 24%, 42%, 62%, 83%, 91%, 98% and 100% for each dose respectively. After washing the preparations, giving them a rest of 30 minutes, and adding a fixed dose of 1µgm of Pancuronium the percent responses were 1%, 1%, 9%, 19%, 47%, 75%, 90%, and 96% for each dose respectively. Semi log dose response curve were plotted by taking the percent responses which showed that the second curve i.e., after washing with frog-Ringer solution and rest and adding 1µg of Pancuronium shifted to the right and downwards. The deviation started with the 1st dose of 1 µg and continued in almost the entire extent of the curve, minimizing at the higher doses. Percentage deviation was calculated for each dose and was 88%, 94%, 79.7%, 69.0%, 43.1%, 17.3%, 8.0% and 4.3% respectively with mean 50.4%. (SEM ± 15.17). P < 0.05.

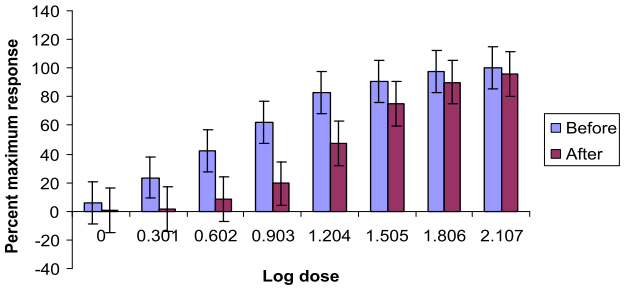

Fig 2: Semi Log Dose Percent Response Bars for Acetylcholine-Induced Contractions, Before and After Adding 1µg Pancuronium

Group III (Ranitidine)

In a series of six experiments the mean ± SEM values of responses for 1, 2, 4, 8, 16, 32, 64, and 128, µg of Acetylcholine were recorded. Percent responses were calculated by taking the response with 128 µg as 100% and were 2%, 9%, 25%, 48%, 66%, 87%, 95%, and 100% for each dose respectively. The same experiment was repeated on the same tissue preparation using similar concentration and doses of Acetylcholine after washing the preparations, giving them a rest of 30 minutes, and adding a fixed dose of 1mM concentration of Ranitidine. The percentage responses with the same doses were 6%, 16%, 47%, 70%, 93%, 111%, 117%, and 117%, for each dose respectively. Semi log dose response curves were plotted by taking the percent responses which showed that the second curve i.e., after washing with frog-Ringer solution and rest and adding 1µM Ranitidine on an average shifted to the left and upwards. The deviation started with the 1st dose and continued in the entire extent of the curve. Percentage deviation was calculated for each dose and was 166%, 83%, 90.9%, 44.6.%, 42.0%, 25.6%, 22.7%, and 17.2% respectively with mean 61%. (SEM ± 20.59). P < 0.05

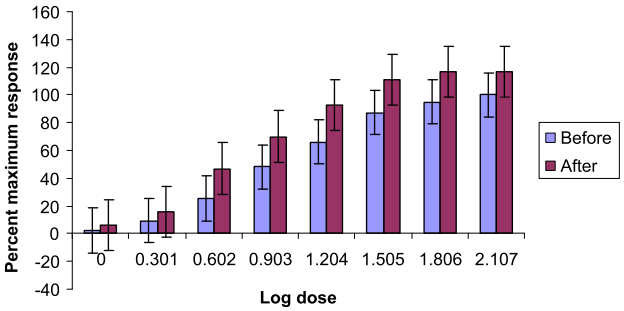

Fig 3: Semi Log Dose Percent Response Bars for Acetylcholine-Induced Contractions Before and After Adding 1mm Ranitidine

Group IV (Pancuronium+ Ranitidine)

In a series of six experiments the mean ± SEM values of responses for 1, 2, 4, 8, 16, 32, 64, and 128 µg of acetylcholine were recorded. Percent responses calculated by taking response with 128 µg as 100% and were 1%, 6%, 15%, 30%, 57%, 89%, 95%, and 100% for each dose respectively. After washing the preparations and adding a fixed dose of 1µg Pancuronium and 1mM Ranitidine the percent responses were 3.2%, 11%, 37%, 71%, 97%, 114%, 120%, and 121% for each dose respectively. Semi log dose response curve were plotted by taking the percentage responses which showed that the second curve i.e., after washing with frog-Ringer's solution and rest and adding Pancuronium and Ranitidine shifted to the left and upwards. The deviation started after the 2nd dose and continued in the entire extent of the curve. Percentage deviation was calculated for each dose and was 312.5%, 100.86%, 142.4%, 135.3%, 69.8%, 28.4%, 25.6%, and 21.1% with mean 104.5%. (SEM ± 39.7). P < 0.05.

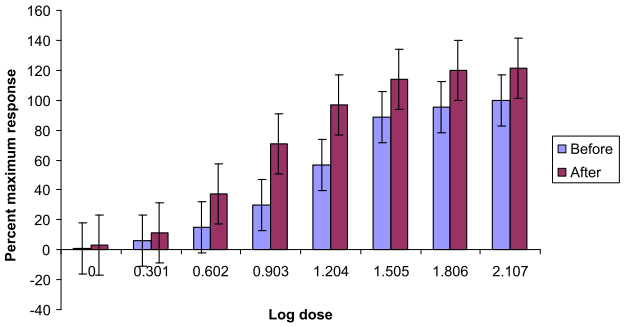

Fig 4: Semi Log Dose Percent Response Bars for Acetylcholine-Induced Contractions, Before and After Adding 1µg Pancuronium and 1mm Ranitidine

Comparison of Control Group I (Acetylcholine) and Group III (Acetylcholine + Ranitidine).

The difference in the percent deviation of response to acetylcholine in the two groups. Fig 5 (P < 0.05).

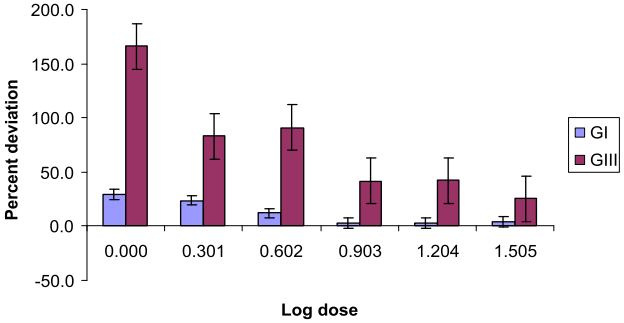

Fig 5: Comparison of Percent Deviation of Group I (Acetylcholine) With Percentage Deviation of Group III (Ranitidine)

Comparison of Group II (Acetylcholine + Pancuronium) and Group IV (Acetylcholine + Pancuronium + Ranitidine)

The difference in the percent deviation of response to acetylcholine in the two groups Fig 6 (P < 0.05).

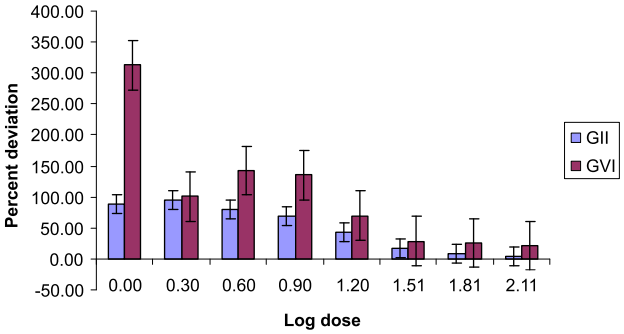

Fig 6: Comparison of Percent Deviation of Group II (Acetylcholine + Pancuronium) and Group IV (Acetylcholine + Pancuronium + Ranitidine)

Comparison of Group II (Acetylcholine + Pancuronium) and Group IV (Acetylcholine + Pancuronium + Ranitidine)

The difference in the percent deviation of response to acetylcholine in the two groups Fig 6 (P < 0.05).

Discussion

In the present study Ranitidine in a dose of 1mM concentration produced a shift of the curve to the left with mean deviation of 61.5% (SEM ± 20.5) showing an enhancement of effects of Acetylcholine.

Ranitidine also produced a shift of the curve to the left in the presence of 1µg Pancuronium with the mean deviation of 104.5% (SEM ± 39.7). The shift was statistically significant (P < 0.05) showing the antagonistic effect of Ranitidine on neuromuscular junction (NMJ) blocker Pancuronium at this concentration.

The difference in the percent deviation of response to Acetylcholine in the two groups (control group I and group III) was highly significant (P < 0.05).

The difference in the percent deviation of response to Acetylcholine in the two groups (group II and group IV) was also highly significant (P < 0.05).

This was consistent with previous studies by Mishra and Kounenis and a clinical case report of resistance to non-depolarizing blocking agents in a patient on a prolonged treatment with Ranitidine.[20,21,22]

This reversal is concentration dependent and is seen only at lower doses while at higher doses the effect is reversed and ranitidine enhances the effects of NMJ blockers possibly by its ion channel blocking

activity.[20] Ranitidine induced reversal of the effects of Pancuronium may be due to the anti-cholinesterase activity of the drug whereby it enhances the effects of Acetylcholine by interfering with its metabolism.[15] This is further reinforced by the fact that Ranitidine does not enhance the tissue responses and conversely shifts the curve to right when Carbachol is used instead of Acetylcholine. Carbachol is resistant to hydrolysis by cholinesterase[23] and in such situations Ranitidine decreases the response to Carbachol in dose range of 0.25mM to 01mM.[24] This NMJ blocking activity of Ranitidine may be due to direct blockade of the ion channel independent of its anti-cholinesterase inhibiting activity.[22] Due to this effect of Ranitidine with Carbachol, it can be inferred that the anti-cholinesterase activity of Ranitidine is relatively stronger as compared to its ion channel blocking properties. Moreover, in actual clinical situations it is Acetylcholine which serves as neurotransmitter at neuromuscular junction, consequently this antagonistic effect of Ranitidine may have important interactions with NMJ blockers in anaesthetic practice.

Conclusion

The present study concludes that Ranitidine in a concentration of 1mM increases the effects of acetylcholine at NMJ and antagonize the effects of NMJ blocker Pancuronium at this concentration.

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