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ORIGINAL ARTICLE

Inhibition of Effects of Acetylcholine at Neuromuscular Junction by Omeprazole

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ABSTRACT

Aim: To assess the effect of Omeprazole 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, Rawalpindi from October 2018 to September 2019
Methods: In this experimental study both a student's oscillograph and a cumulative dosage response curve were used to record the frog's rectus abdominis muscle contracting in response to acetylcholine (control group). Three groups' responses to omeprazole, both before and after adding pancuronium, were noted. The student's t test was used to conduct statistical analysis of variance (ANOVA) between the groups, and a p-value of 0.05 or below was regarded as statistically significant.
Results: By blocking the neurotransmitter acetylcholine, omeprazole in a dose of 3.5 M concentration altered the curve to the right with a mean deviation of 23.7% (SEM ± 20.5). In the presence of 1 g of pancuronium, omeprazole likewise caused a shift of the curve to the right with a mean deviation of 37 percent (SEM ±10.5). The change demonstrated the agonistic impact of omeprazole on NMJ blockers like pancuronium at this concentration and was statistically significant ($p < 0.05$).
Conclusion: Omeprazole in 3.5µM strength inhibits the effects of Acetylcholine at neuromuscular junction, however, it potentiates the effects of neuromuscular blockers like Pancuronium at same strength.
Keywords: Acetylcholine**,** Neuromuscular Junction, Pancuronium, Omeprazole.

INTRODUCTION

Proton pump inhibitors like omeprazole are broadly used worldwide to treat gastricacid hypersecretory states including acid peptic disease, Helicobacter pylori (H. Pylori) and Zollinger Ellison syndrome[1].Proton pump inhibitors reduce the gastric acid secretion by targeting the H^+/K^+ pump of the gastric parietal cells[2].Aspiration pneumonia caused by regurgitation of gastric juice during general anesthesia is always a challenge for the anesthesiologist[3]. H_2 receptor antagonists and proton pump inhibitors are given preoperatively for the prophylaxis of gastric acid aspiration[4,5].

Daily 30 to 40 million patients receive general anesthesia for various surgical procedures.To achieve immobility during the procedure neuromuscular blocking agents are given along with other anesthetic agents. Non depolarizing neuromuscular junction (NMJ) blockers like pancuronium, vecuronium and rocuronium are administered before tracheal intubation and intraoperatively for muscle relaxation. Site of action of these drugs is the NMJ.Non depolarizing muscle relaxants competitively inhibit the binding of the neurotransmitter acetylcholine to postsynaptic receptors located at the muscular end of NMJ,causing paralysis of the muscle[6–8].

Histamine H_2 receptor antagonists enhance the neuromuscular blocking action of pancuronium by potentiating the effect of acetylcholine at NMJ[9]. Proton pump inhibitors lessen the lower esophageal contractions[10]. Neuromuscular transmission is impeded by proton pump inhibitors.Lansoprazole when used as a component of premedication before anesthesia enhances the neuromuscular block produced by vecuronium in humans[11]. Omeprazole potentiates the paralysis produced by atracurium and succinylcholine in rodents[11].It was therefore pertinent to determine the activity of Omeprazole at NMJ and its interaction with NMJ blockers like Pancuronium.

The current experiment was executed to assess the effect of Omeprazole at NMJ with and without the effect of Pancuronium at NMJ.

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MATERIALS AND METHODS

This is an experimental study that wasconducted in the Department of Pharmacology and Therapeutics, Islamic International Medical College, Rawalpindi after obtaining approval from ethical review committee of the same institute.Twenty-four adult *Rana tigrine* frogs of either gender, weighing 100 to 150 grams were selected and divided in to four different groups randomly. Rectus abdominis muscles from allfrogs were isolated after dissection and kept in frog ringer solution at room temperature and aerated continuously with 100% oxygen.Muscular contractionson oscillograph via isotonic transducer were taking for different drugs after mounting a single tissue in organ bath.

In group A, Cumulative dose response curve was recorded for various cumulative doses of Acetylcholine (ACh) from 2µg to 32µg and labelled as control. In group B, Cumulative dose response curve for various cumulative doses of Ach before and after 1µg Pancuronium was obtained. In group C, similar readings were obtained with AChas in group B but with 3.5µM solution of Omeprazole. In group D, cumulative dose response curve with ACh was recorded before and after Pancuronium and Omeprazole for every preparation.

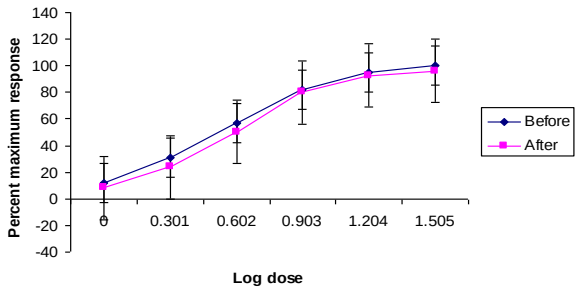
The student's t test was used to do statistical comparison between the groups, and a p-value of 0.05 or below was deemed statistically significant.

RESULTS

Responses in group A were 12%, 31%, 57%, 82%, 95%, and 100% for doses of 1, 2, 4, 8, 16, and 32 µg of ACh, respectively. However, the responses for each dose were 8%, 24%, 50%, 80%, 92% and 96% after drug wash and break of 30 minutes. By using the percentage responses, semi-log dosage response curve was constructed using percentage responses (taken after drug wash) was shifted to the right and downwards.For each dose, the percent deviation was calculated, and the results were 29.4%, 23.94%, 11.94%, 2.54%, 2.94%, and 4.14%, respectivelywith mean value of 12.45 (SEM± 4.77) (Fig. I).

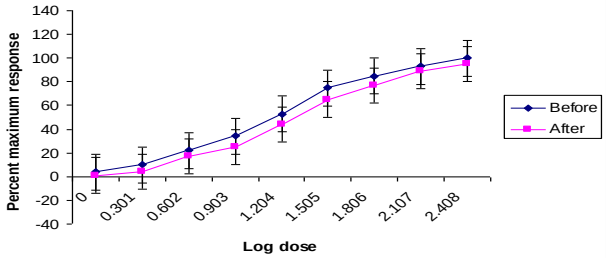
In Group B, responses were 6%, 24%, 42%, 62%, 83%, 91%, 98% and 100%for doses of 1, 2, 4, 8, 16, 32, 64, and 128 µg of ACh, respectively. However, the responses for each dose were 1%, 1%, 9%, 19%, 47%, 75%, 90%, and 96% after drug wash and break of 30 minutes and adding 1µg of Pancuronium. By using the percentage responses, semi-log dosage response curve was constructed using percentage responses (taken after drug wash and treatment with 1µg of Pancuronium) was shifted to the right and downwards. For each dose, percent deviation was calculated, and the results were 88%, 94%, 79.7%, 69%, 43.1%, 17.3%, 8.0% and 4.3% respectively with mean 50.4 (SEM±15.17) (Fig. II)

Figure-I: Cumulative log dose percent response curves for ACh-induced contractions, before and after washing with frog-Ringer solution (Group A)



In Group C, responses for nine cumulative doses of ACh that is from 1 to 256 µgwere calculated to be 4%, 10%, 22%, 34%, 53%, 75%, 85% 93%, and 100% for each cumulative dose respectively. However, the responses for each dose 1%, 4%, 17%, 25%, 44%, 65%, 77%, 89%, and 95% after drug wash and break of 30 minutes and adding 3.5µM of Omeprazole. By using the percentage responses, semi-log dosage response curve wascharted down using percentage responses (taken after drug wash and treatment with 3.5µM Omeprazole) was shifted to the right and downwards with prominent central portion.For each dose, the percent deviation was calculated, and the resultswere60.2%, 53.7%, 24%, 26.3.%, 16.7%, 12.9%, 9.7%, and 4.7%and 4.9% respectively with mean 23.7% (SEM ± 8.35).(Figure-III).

Figure-III: (ACh + Omeprazole) Cumulative log dose percent response curves for ACh-induced contractions Pre- and post-treated with 3.5µM omeprazole (Group c)



In Group D, responses for nine cumulative doses of Ach were recorded as in group Cfrom 1 to 256 µg dose. The percentage response of nine doses of Ach were 8%, 14%, 20%, 29%, 42%, 54%, 69%,93% and 100%for each cumulative dose respectively. However, the responses for each dose 2%, 7%, 8%, 16%, 24%, 44%, 62%, 80% and 90%after drug wash and rest of 30 minutes and adding1µg Pancuronium and3.5µM of Omeprazole. By using the percentage responses, semi-log dosage response curve wascharted down using percentage responses (taken after drug wash and treatment with 1µg Pancuronium and 3.5µM Omeprazole) was shifted to the right and downwards.For each dose, the percent deviation was calculated, and the resultswere75.0%, 68.6%, 58.6%, 45.3%, 33.7%, 17.7%, 10%, 13.7% and 10.2%. with mean 37% (SEM ± 10.59). (Figure-IV)

Comparison of Control Group A (ACh) and Group C (ACh + Omeprazole): Percent deviations produced in Control Group A with doses of 1, 2, 4, 8, 16, and 32µg of AChwere 29.4, 23.9, 11.9, 2.5, 2.9, and 4.1 % respectively. The mean deviation was 12.5 percent ± 4.7. Percent deviations produced with the same doses of ACh in the group C were 60.2, 53.7, 24, 26.3, 16.7, and 12.9 percent respectively. The mean deviation was 32.3 percent ± 8.11. The p value comparing the two groups was < 0.05. (Fig.-V). The difference between the two groups' deviations in response to ACh was statistically significant (p 0.05).

Figure-IV: (ACh + Pancuronium + Omeprazole) Semi log dose percent response curves for ACh-induced contractions Pre- and post-treated with 1µg pancuronium and 3.5µM omeprazole (Group D)

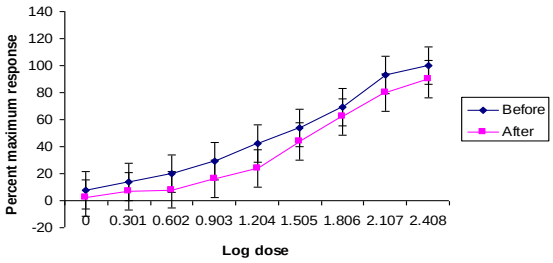
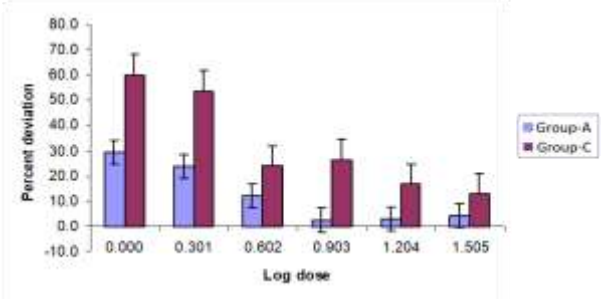
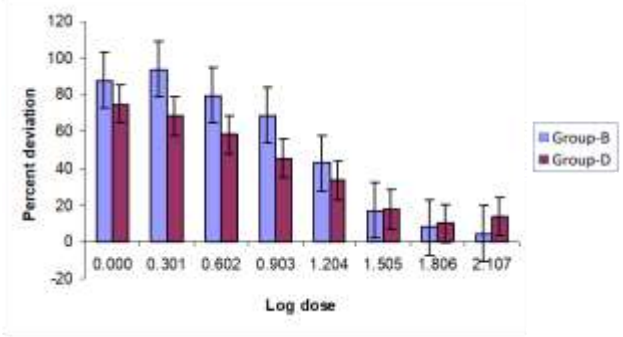


Figure-V: Comparison of percent deviation of group A (ACh) with percentage deviation of group C (Omeprazole)



Comparison of Group B (ACh + Pancuronium) and Group D (ACh + Pancuronium + Omeprazole): Percent deviations produced in Group B with doses of 1, 2, 4, 8, 16, 32, 64, and 128µg of ACh were 88, 94, 79.7, 69, 43.1, 17.3,8, and 4.3 % respectively. The mean deviation was 50.4 percent. ± 15.17. Percent deviations produced with the same doses in the group D were 75, 68.6, 58.6, 45.3, 33.7, 17.7, 10, and 13.7 % respectively. The mean deviation was 40.33 percent ± 10.44. (Figure-VI).The difference between the two groups' deviations in response to ACh was statisticallysignificant (p 0.05).

Figure-VI: Comparison of percent deviation of group B (ACh + Pancuronium) and Group D (ACh + Pancuronium + Omeprazole)



DISCUSSION

In the current experiment omeprazole in 3.5µM strength shifted the curve to the right especially in the middle portion of the curve and the mean deviation was 23.7%±8.35 S.E.M which was statistically significant. This rightward shift was also present when the experiment was repeated by adding 1µg pancuronium as a constant dose. The statistically significant average deviation of 40.33%±10.44 S.E.M was observed.

These findings when are compared with the control Group A& Group B show that the right deviation seen with omeprazole is significant statistically with P <0.05. Thus showing that Omeprazole itself has NMJ blocking properties and it also enhances the NMJ blocking properties of NMJ blockers like Pancuronium.

These findings are consistent with the findings of Mishra and Ramzan that showed that omeprazole when used in rats in *vivo* enhanced the effects of both atracurium and succinylcholine[12]. Enhancement of effect of succinylcholine by omeprazole is also documented by its greater ability to inhibit cholinesterase and increasing the levels of ACh especially in Phase I block[13].Cholinesterase inhibiting ability of omeprazole has also been documented in a study by Margarida Ramos and others[14]. Similarly, pantoprazole-induced neuromuscular blockade has also been documented[15].

The reason for enhancing the NMJ blocking activity of both depolarizing and non-depolarizing blockers are rather perplexing because if it was due to the cholinesterase inhibition property of omeprazole[13],then it should enhance the effects of depolarizing blockers and antagonize the action of non-depolarizing blockers. As only a single dose was used in this study perhaps further investigations are required at other concentrations as well.

CONCLUSION

Our current experiment showed that the omeprazole in 3.5µM strength decreases the effects of ACh at NMJ of frog's rectus abdominis muscle and also enhances the activity of NMJ blockers like pancuronium at same strength.

Conflict of interest: Nothing to declare

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