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TO COMPARE THE INHIBITORY EFFECTS OF VERAPAMIL WITH LOPERAMIDE ON ACETYLCHOLINE INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

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ABSTRACT

BACKGROUND: *Fundamental and an essential step for the cell contraction is an increase in cytosolic free calcium concentration. The increase in calcium concentration occurs by influx from the extra cellular medium through calcium ion channels and by calcium release from the rapidly exchanging intracellular stores. Voltage operated calcium channels have potential role in regulation of motility in gastrointestinal tract, so there is growing interest in calcium channel blocker as a potential pharmacological approach to the treatment of various gastrointestinal motor disorders. In this study we evaluate and compare the inhibitory effects of Verapamil with Loperamide on acetylcholine induced contractions of isolated guinea pig ileum.*

MATERIALS AND METHODS: *Isolated s.intestine of twenty four guinea pigs was used. Serial dilutions of Loperamide and Verapamil were administered in the presence of Acetylcholine (10^{-6}gm/ml), effects observed and recorded by 7B Grass Polygraph machine.*

RESULTS: *We observed that at lower concentrations Loperamide showed more inhibitory effects than Verapamil, while at higher concentrations (10^{-4} and 10^{-3} gm/ml) Verapamil showed more inhibitory effects than Loperamide.*

CONCLUSION: *We found that Verapamil is a potent inhibitor of small intestinal contractions as Loperamide. However one can presume further that calcium channel blocker Verapamil acting on calcium channels of GIT will be developed with more specific effect on smooth muscle of intestinal tract.*

KEY WORDS: Verapamil, Loperamide, Acetylcholine and Ileum.

INTRODUCTION

Smooth muscles composed of smaller fibers, usually 1-5 micrometers in diameter and 20-500 micrometers in length. Smooth muscles in many tissues are capable of partial contraction almost indefinitely to regulate the diameter of hollow organs[6]. Smooth muscle cells contain large amount of regulatory proteins called calmodulin. Calmodulin is a calcium dependent regulatory protein that mediates regulation of numerous calcium dependent intracellular events. In smooth muscle in the presence of calcium, a complex is formed between calcium, calmodulin and myosin light-chain kinase (MLCK), which then phosphorylates the myosin light chain called regulatory chain and initiates cross-bridge cycling and contraction[3, 7].

To activate the contractile apparatus, Ca^{2+} must increase globally throughout the cytoplasm. The Ca^{2+} utilized for activation of the contractile apparatus enters the cytoplasmic compartment during periods of membrane depolarization, mechanical distortion, or stimulation by agonists. Release of Ca^{2+} from intracellular stores is a second means of increasing $[Ca^{2+}]_i$. After an excitatory event, relaxation and Ca^{2+} homeostasis are achieved by reuptake of Ca^{2+} into stores and extrusion into the extra cellular space[13]. To cause relaxation of smooth muscles, it is necessary to remove the calcium ions from the intracellular fluids surrounding the actin myosin filaments. This removal is achieved by a calcium pump that pumps the calcium ions out of the smooth muscle fibers back into the extra cellular fluid or pumps calcium ions into the sarcoplasmic reticulum[6].

All electrically excitable cells through out the animal kingdom possess Ca^{2+}-channels[14]. The blockade of L-type Ca^{2+} channels by Verapamil significantly inhibits the frequency

of giant migrating contractions (GMC) during small intestinal inflammation[10].

Loperamide is a piperidine derivative. Its mechanism of action is as of opioids[1]. The chemical structure of Loperamide resembles organic calcium channel antagonist drugs such as Verapamil. Although calcium channel antagonist drugs are used predominantly for cardiovascular disorders, they do affect other tissues and organs. Constipation is a major side effect of treatment with calcium antagonists such as Verapamil. Loperamide displays pharmacological calcium antagonism in smooth muscle. Their calcium antagonists' actions may be responsible in part for antidiarrheal effects. The intestinal concentration of Loperamide after pharmacologically relevant doses is sufficient to block calcium channels. It is believed that the control of electrolyte and fluid secretion is involved both in etiology of diarrhea and its control by opiates. Calcium regulates the balance between absorption and secretion across the intestinal mucosa. Low intracellular calcium levels favor absorption; rise in intracellular calcium promotes secretion[12]. Acetylcholine is the major excitatory neurotransmitter to the smooth muscle cells of intestine. It activates contraction through an increase in the concentration of Ca^{2+} in the cytosol[2, 9].

PURPOSE OF STUDY

"To evaluate and compare the inhibitory effects of calcium channel blocker Verapamil with opioid drug Loperamide on Acetylcholine induced contractions of isolated guinea pig ileum".

STUDY DESIGN

Duration of time:

All experimental works were carried out in the Department of Pharmacology and Therapeutics, Basic Medical Science Institute (B M S I), Jinnah Post Graduate Medical Center (J P M C) Karachi. Duration of time taken for all experiment work was eight weeks.

MATERIALS AND METHODS:

Drugs Verapamil and Loperamide were supplied from ZAFA pharmaceutical Pakistan limited Karachi. While Acetylcholine was supplied by BMSI, JPMC, Karachi

Serial dilutions of all drugs were made from 10^{-3} gm/ml to 10^{-18} gm/ml.

Nutritional Solution:

Oxygen and the specified Tyrode physiological nutrient solution (contains Sodium Chloride, Potassium Chloride, Calcium Chloride Anhydrous, Magnesium

TABLE (A): COMPARISON OF LOPERAMIDE AND VERAPAMIL IN GROUP II & III ON THE BASIS OF AMPLITUDE OF CONTRACTION.

Drug	Concentration (gm/ml)	Acetylcholine Rate Mean ± S.E.M	Drug Rate Mean ± S.E.M	Decrease (%)
Loperamide	10^{-18}	19.25 ± 2.74	14.38 ± 2.6	25.32
Verapamil	10^{-18}	24.00 ± 1.65	21.00 ± 1.6	12.50
	p - value	N.S. P<0.01		
Loperamide	10^{-17}	19.75 ± 2.19	13.75 ± 2.4	30.38
Verapamil	10^{-17}	26.50 ± 1.49	22.88 ± 1.6	13.68
	p - value	N.S. p<0.001		
Loperamide	10^{-16}	21.88 ± 3.23	15.88 ± 2.4	27.43
Verapamil	10^{-16}	26.25 ± 2.11	22.25 ± 2.0	15.24
	p - value	N.S. N.S.		
Loperamide	10^{-15}	24.13 ± 2.26	16.88 ± 2.7	30.05
Verapamil	10^{-15}	25.00 ± 2.10	20.63 ± 1.9	17.50
	p - value	N.S. N.S.		
Loperamide	10^{-14}	25.25 ± 3.03	19.63 ± 2.8	22.28
Verapamil	10^{-14}	26.00 ± 1.74	21.25 ± 1.6	18.27
	p - value	N.S. N.S.		
Loperamide	10^{-13}	23.88 ± 2.75	18.38 ± 2.6	23.04
Verapamil	10^{-13}	25.13 ± 1.08	19.88 ± 1.1	20.90
	p - value	N.S. N.S.		
Loperamide	10^{-12}	23.63 ± 3.14	17.13 ± 3.3	27.51
Verapamil	10^{-12}	26.13 ± 0.95	20.38 ± 1.2	22.01
	p - value	N.S. N.S.		
Loperamide	10^{-11}	24.13 ± 2.52	16.50 ± 2.5	31.61
Verapamil	10^{-11}	25.38 ± 1.72	20.13 ± 1.2	20.69
	p - value	N.S. N.S.		
Loperamide	10^{-10}	23.00 ± 2.61	16.00 ± 2.3	30.43
Verapamil	10^{-10}	24.88 ± 1.78	19.63 ± 1.7	21.11
	p - value	N.S. N.S.		
Loperamide	10^{-9}	22.50 ± 2.47	14.88 ± 2.1	33.89
Verapamil	10^{-9}	26.25 ± 1.29	20.13 ± 1.2	23.33
	p - value	N.S. p<0.01		
Loperamide	10^{-8}	22.38 ± 2.24	13.75 ± 2.4	38.55
Verapamil	10^{-8}	26.00 ± 1.16	20.00 ± 1.4	23.08
	p - value	N.S. p<0.01		
Loperamide	10^{-7}	22.88 ± 2.47	13.75 ± 2.7	39.89
Verapamil	10^{-7}	26.88 ± 1.54	20.13 ± 1.6	25.12
	p - value	N.S. N.S.		
Loperamide	10^{-6}	22.75 ± 2.24	15.00 ± 2.5	34.07
Verapamil	10^{-6}	25.50 ± 1.71	18.25 ± 1.8	28.43
	p - value	N.S. N.S.		
Loperamide	10^{-5}	24.50 ± 1.61	16.25 ± 2.4	33.67
Verapamil	10^{-5}	24.88 ± 2.78	16.25 ± 3.1	34.67
	p - value	N.S. N.S.		
Loperamide	10^{-4}	23.88 ± 1.86	17.88 ± 2.5	25.13
Verapamil	10^{-4}	21.25 ± 3.16	5.75 ± 1.9	72.94
	p - value	N.S. p<0.001		
Loperamide	10^{-3}	24.38 ± 2.03	19.00 ± 2.2	22.05
Verapamil	10^{-3}	12.75 ± 2.28	2.38 ± 1.8	81.37
	p - value	p<0.01p<0.001		

Chloride, Sodium Bicarbonate, Sodium Dihydro Phosphate, Dextrose, and Distilled Water) was used for the perfusion of isolated intestinal segments.

Preparation and Isolation of Small Intestine:

Twenty four healthy adult guinea pigs of both sexes (non pregnant) were used, obtained from animal house of J P M C Karachi, weighing 450 – 750 gm. The animals were killed by a blow on the head with hammer. The abdomen was opened by midline incision and ileum was located. A piece of terminal ileum was separated and transferred to Petri dish containing aerated (oxygenated) Tyrode solution, where it was cleaned from fecal material, fat and extra connective tissues. A segment of about 15 – 20 mm long was taken from isolated ileum, and mounted vertically in inner organ bath (which contains 20 ml Tyrode solution) with the help of tissue holder. It was connected to Polygraph with the help of force displacement transducer, and was subjected to the tension of 1 gm. The nutrition solution was continuously aerated. Temperature of organ bath was maintained at 37°C. Preparations were allowed to equilibrate in Tyrode solution for at least 30 – 45 minutes. The drugs were added in small quantities (1 ml) to inner organ bath according to experimental protocol.

Methodology:

Eight experiments were done in each group. In group-I, the tissues were exposed to serial dilutions (from10^{-18} to 10^{-3} gm/ml) of Acetylcholine and responses were recorded for two minutes for each dilution respectively. From those responses, standard (control) concentration of Acetylcholine was selected (i.e. 10^{-6}gm/ml) which had produced maximum response. While in group-II and III, in the presence of standard (control) concentration of Acetylcholine the tissues were challenged with serial dilutions (from 10^{-18} to 10^{-3} gm/ml) of Loperamide and Verapamil respectively and responses were recorded by Polygraph again for two minutes for each dilution respectively.

OBSERVATIONS AND RESULTS

Results were based on the rate and amplitude of Acetylecholine induced
contractions of isolated ileum recorded by Polygraph with the help of force displacement transducer.

PERCENT CHANGES

When we compare percent changes of D1 (Loperamide) and D2 (Verapamil), we found that at lower concentration D1 was more

TABLE (A): COMPARISON OF LOPERAMIDE AND VERAPAMIL IN GROUP II & III ON THE BASIS OF RATE OF CONTRACTION.

Drug	Concentration (gm/ml)	Acetylcholine Amplitude (mm) Mean ± S.E.M	Drug Amplitude (mm) Mean ± S.E.M	Decrease (%)
Loperamide	10^{-18}	184.25 ± 29.11	141.38 ± 22.78	23.27
Verapamil	10^{-18}	136.00 ± 21.06	110.75 ± 17.10	18.57
	p - value	N.S.	N.S.	
Loperamide	10^{-17}	157.13 ± 31.30	106.25 ± 18.62	32.38
Verapamil	10^{-17}	151.25 ± 17.19	122.75 ± 13.87	18.84
	p - value	N.S	N.S.	
Loperamide	10^{-16}	188.25 ± 41.99	136.88 ± 32.99	27.29
Verapamil	10^{-16}	163.88 ± 24.59	121.38 ± 19.33	25.93
	p - value	N.S.	N.S.	
Loperamide	10^{-15}	242.88 ± 71.96	147.75 ± 37.10	39.17
Verapamil	10^{-15}	154.38 ± 22.96	116.63 ± 19.48	24.45
	p - value	N.S.	N.S.	
Loperamide	10^{-14}	252.63 ± 60.29	162.13 ± 35.15	35.82
Verapamil	10^{-14}	172.13 ± 25.95	121.00 ± 21.93	29.70
	p - value	N.S. N.S.		
Loperamide	10^{-13}	274.50 ± 56.70	171.00 ± 38.28	37.70
Verapamil	10^{-13}	171.25 ± 31.02	118.50 ± 27.38	30.80
	p - value	N.S.	N.S.	
Loperamide	10^{-12}	295.50 ± 59.21	193.25 ± 38.02	34.60
Verapamil	10^{-12}	190.88 ± 32.19	140.75 ± 28.98	26.26
	p - value	N.S.	N.S.	
Loperamide	10^{-11}	277.13 ± 46.02	197.38 ± 41.90	28.78
Verapamil	10^{-11}	197.00 ± 33.81	147.38 ± 31.78	25.19
	p - value	N.S.	N.S.	
Loperamide	10^{-10}	296.13 ± 47.82	205.13 ± 36.62	30.73
Verapamil	10^{-10}	220.50 ± 40.17	153.13 ± 31.82	30.56
	p - value	N.S.	N.S.	
Loperamide	10^{-9}	318.50 ± 53.06	205.25 ± 28.68	35.56
Verapamil	10^{-9}	206.38 ± 24.91	141.00 ± 20.02	31.68
	p - value	N.S.	N.S.	
Loperamide	10^{-8}	341.38 ± 60.54	227.38 ± 41.36	33.39
Verapamil	10^{-8}	206.13 ± 26.59	135.88 ± 20.05	34.08
	p - value	N.S.	N.S.	
Loperamide	10^{-7}	291.50 ± 57.00	175.00 ± 40.60	39.97
Verapamil	10^{-7}	232.75 ± 19.50	170.88 ± 17.05	26.58
	p - value	N.S.	N.S.	
Loperamide	10^{-6}	331.50 ± 59.28	193.25 ± 39.87	41.70
Verapamil	10^{-6}	233.50 ± 16.59	145.13 ± 16.68	37.85
	p - value	N.S.	N.S.	
Loperamide	10^{-5}	326.38 ± 55.88	194.25 ± 44.82	40.48
Verapamil	10^{-5}	208.50 ± 28.62	97.13 ± 21.28	53.42
	p - value	N.S.	N.S.	
Loperamide	10^{-4}	326.38 ± 55.88	178.63 ± 49.07	45.27
Verapamil	10^{-4}	208.50 ± 28.62	9.88 ± 3.65	95.26
	p - value	N.S.	p<0.001	
Loperamide	10^{-3}	251.38 ± 83.63	210.88 ± 80.91	16.11
Verapamil	10^{-3}	32.50 ± 8.19	3.25 ± 2.16	90.00
	p - value	p<0.01	p<0.01	

decreasing the rate, while the amplitude was more decreased by D2, but at higher concentrations (10^{-4} gm/ml and 10^{-3} gm/ml) D2 was more decreasing the rate as well as amplitude of Acetylecholine induced contraction than D1.

STATISTICALLY

***Rate*:**

When D1 and D2 were compared on the basis of rate of contraction

after exposure to Acetylcholine, there was significant ($p<0.01$) difference observed at lower concentration. That difference was due to smaller mean values of D1 than D2, while at higher concentrations (10^{-4} gm/ml and 10^{-3}gm/ml) highly significant difference was found with p-value $p<0.001$. That difference was due to smaller mean values of D2 than D1.

***Amplitude*:**

When D1 and D2 were compared on the basis of amplitude of contraction in isolated ileum, after exposure to Acetylcholine, there was non significant difference observed with smaller mean value of D2 than D1. But when we compared D1 and D2 at higher concentration (10^{-4}gm/ml and 10^{-3}gm/ml) a highly significant difference was seen with p-values $p<0.001$. That difference was due to smaller mean values of D2 than D1. [Table no. (a) and (b)]

TABLE (A): COMPARISON OF LOPERAMIDE AND VERAPAMIL IN GROUP I & II ON THE BASIS OF RATE OF CONTRACTION.

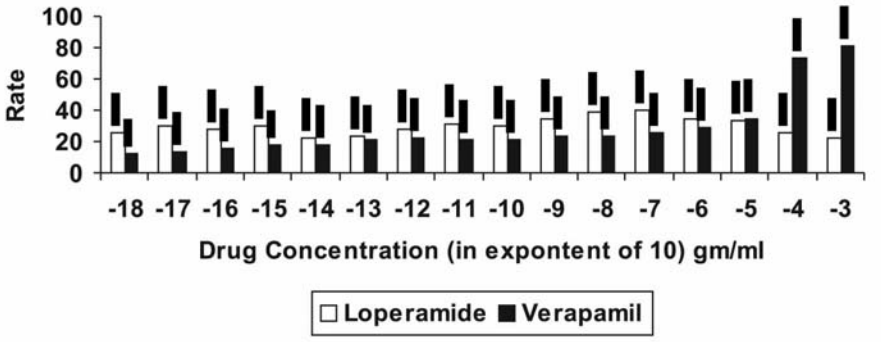
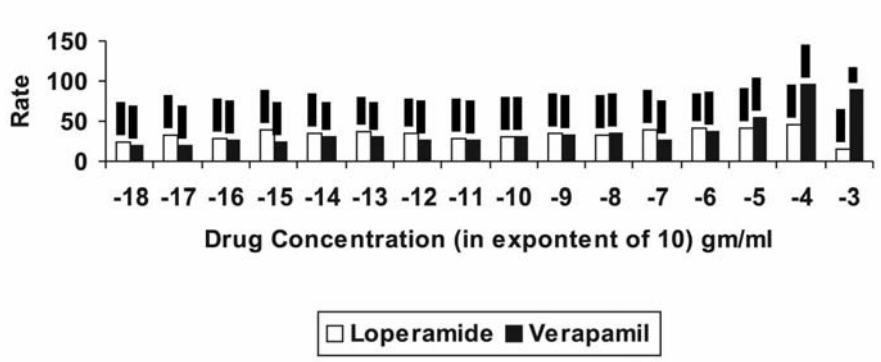


TABLE (B): COMPARISON OF LOPERAMIDE AND VERAPAMIL IN GROUP I & II ON THE BASIS OF AMPLITUDE OF CONTRACTION.



DISCUSSION

Our objective for this study was not to review all that is known about the pharmacology of these drugs (Loperamide and Verapamil) but to investigate a hypothesis for the inhibitory role played by calcium channel blocker Verapamil in comparison with Loperamide, on Acetylcholine induced motility of ileum.

Daly J. W and Harper J said in his study that Loperamide is widely used as antidiarrheal that primarily acts at nano molar concentration through activation of opioid receptors in the gastro intestinal tract. At some what higher concentrations Loperamide blocks (calmodulin activity) calcium channels. Loperamide at micro molar concentration had now been shown to have a remarkable stimulatory effect on the capacitative calcium influx[5]. Our observations were in complete agreement with these responses of Loperamide that at lower concentrations (10^{-9} gm/ml) Loperamide decreased the rate and amplitude non-significantly, whereas at somewhat higher concentration (10^{-8} gm/ml) Loperamide had significantly decreased the rate as well as amplitude (may be due to calcium channel blocking action) with p-values $p<0.01$, while at mili molar (10^{-3} gm/ml) concentration Loperamide showed not increase in rate and amplitude of contraction but only its inhibitory response decreased (it may be due to increase influx of calcium). In this way Chandra R, studies that in the concentration range (10, 50, 100 and 150 µ mol) papaverine caused relaxation of rhythmic contractions (7.80 ± 0.27 percent, 26.70 ± 0.32 percent, 43.90 ± 2.72 percent and 68.46 ± 3.49 percent respectively)[4]. We also observed antispasmodic activity in the similarly dose dependent manner with in the concentration range (10^{-18}, 10^{-13}, 10^{-8} and 10^{-3} gm/ml) of Verapamil caused reduction in amplitude (18.57 percent, 30.80 percent, 34.80 percent and 90.00 percent respectively) of Acetylcholine induced contraction in isolated guinea pig ileum. Honda H, found that in calcium free solution, Loperamide and Verapamil had no influence, but markedly inhibited calcium induced contractions[8]. We had also used these two drugs i.e., Loperamide and Verapamil, both significantly decreased the frequency and amplitude of Acetylcholine – induced contractions in calcium containing solution. Ponti F.D also studied the effects of Verapamil in abnormal intestinal motility on conscious dogs. He observed that at lower doses Verapamil had non significant effects different from control. Only spike activity and motility index were reduced in phase II and III, while Verapamil at higher doses suppressed migrating motor complex (MMC) cycling and almost completely abolished both spike and mechanical activities at all sites[11]. Our observations correlates with their findings, as we observed that at lower concentrations Verapamil had non-significant inhibitory effects on Acetylcholine induced contractions while at higher concentration it had highly significant ($p<0.001$) inhibitory effect and completely abolished the small intestinal contractions.

CONCLUSION

From above discussion it has been concluded that Loperamide an opioid derivative as well as Verapamil a calcium channel blocker were found potent inhibitors of Acetylcholine induced contractions of isolated guinea pig ileum. After statistically analysis and percentage wise comparison of Loperamide and Verapamil, we observed that at lower concentrations Loperamide

showed more inhibitory effects than Verapamil while at higher concentrations (10^{-4} gm/ml and 10^{-3} gm/ml) Verapamil showed more inhibitory effects than Loperamide on Acetylcholine induced contractions of isolated guinea pig ileum .“However work remains to establish the inhibitory effects of Verapamil that had seen on isolated guinea pig ileum will confirm on vivo study so human studies are awaited”.

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