MEDICAL CHANNEL

Original Article

IN VITRO EFFECTS OF LOPERAMIDE ON SPONTANEOUSLY CONTRACTING AND PRIMED ILEUM OF GUINEA PIG

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ABSTRACT :

Object: To compare the inhibitory effects of loperamide on spontaneous and acetylcholine induced contractions of isolated ileum of Guinea pig.
Study Design: Comparative controlled in-vitro experimental Study.
Place and Duration of Study: This study was conducted at Department of Pharmacology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC) Karachi for the period of six months.
Materials and Methods: Isolated small intestine of twenty four Guinea pigs was used and fresh Tyrode nutritional solution was prepared for each experiment. They were divided in three groups. In group I, standard concentration of acetylcholine was detected. While in group II ileal spontaneously contractions and in group III acetylcholine primed ileum were exposed to serial dilutions of loperamide (from 10^{-18} to 10^{-3} gm/ml) and effects were observed and recorded by 7B Grass Polygraph .
Results*:* Loperamide inhibits the frequency of spontaneous contractions of ileum ranging from 13.17% to 25.36% and amplitude ranging from 19.74% to 37.74% with different serial dilutions. While loperamide inhibits the frequency of acetylcholine induced contractions of ileum ranging from 25.32% to 39.89% and amplitude ranging from 32.38% to 45.27% with different serial dilutions.
Statistically: We also found significantly ($p<0.05$) difference observations in between group II and III, due to higher inhibitory values in group III.
Conclusion*:* Loperamide was found more potent inhibitor for acetylcholine induced contractions than for the spontaneous contractions of isolated ileum of Guinea pig .

Key Words: Loperamide, Acetylcholine and Small intestinal Movements.

INTRODUCTION

Diarrhoeal diseases are one of the leading causes of childhood morbidity and mortality in developing countries. An estimated 1000 million episodes occur each year in children under 5 year of age, especially in developing countries [11,12, 18].

Among the important chemicals, neurotransmitters and hormones that affect the gastrointestinal contractions are nor-epinephrine, epinephrine, acetylcholine, angiotensin, vasopressin, oxytocin, serotonin and histamine [1]. Giant migrating contractions (GMC) produce frequent mass movements that are associated with diarrhea and abdominal cramps [2]. Ca^{2+} is an important ion required for muscular contractions and regulation of many cellular events [3,4]. It is also involved in cellular transduction pathways and regulation of cellular phenotypes[5]. Calcium is considered as fundamental second messenger that plays an important role in the physiology and pathophysiology of numerous cell types, including gut smooth cells[6]. There are two main sources of Ca^{2+}, namely extra cellular Ca^{2+}, and intracellular Ca^{2+}. Within cell, sarcoplasmic reticulum serves as an important reservoir of Ca^{2+} [7]. In most cases, a rise in intracellular Ca^{2+} is a prerequisite for smooth muscle contraction[8].

Acetylcholine is one of the major excitatory neurotransmitter released by the

parasympathetic nervous system, mediate its actions in the gut by stimulation of M_3 muscarinic receptors subtypes[25], and causes increased contractions of the small intestine[21]. It activates contraction through an increase in the concentration of Ca^{2+} in the cytosol. Acetylcholine activates calcium current not directly but via a membrane depolarization. Here depolarization results from an acetylcholine-induced inward current[9,10].

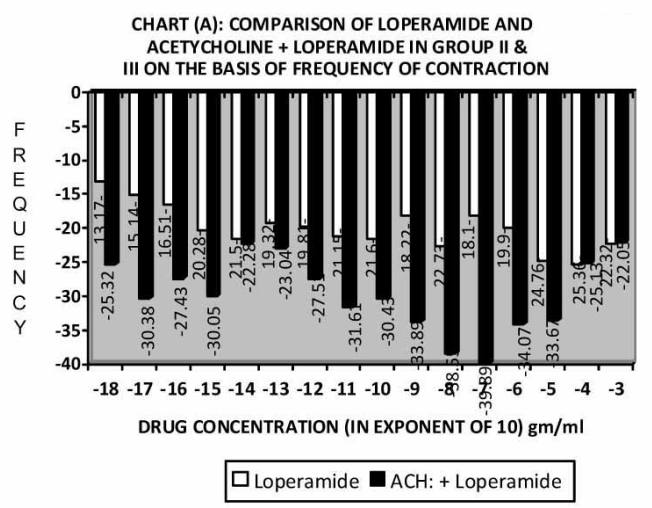
Loperamide, a peripheral opioid receptor agonist[19] that inhibits colonic muscular activity, perhaps partly by interference with release of acetylcholine at the neuroeffector junctions[13]. Loperamide, apart from regulating the gastrointestinal tract, is also reported to abolish experimental osmotic diarrhea by acting on intestinal motility[24], and slow down transit in the intestine, reduce colon flow rates and consequently any effect on colonic motility[18].The chemical structure of loperamide resembles organic calcium channel antagonist drugs such as verapamil. The intestinal concentration of loperamide at therapeutically 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 levels favor absorption; rise in intracellular calcium promotes secretion[20]. Loperamide also caused the inhibition of spontaneous contractions [15,23].

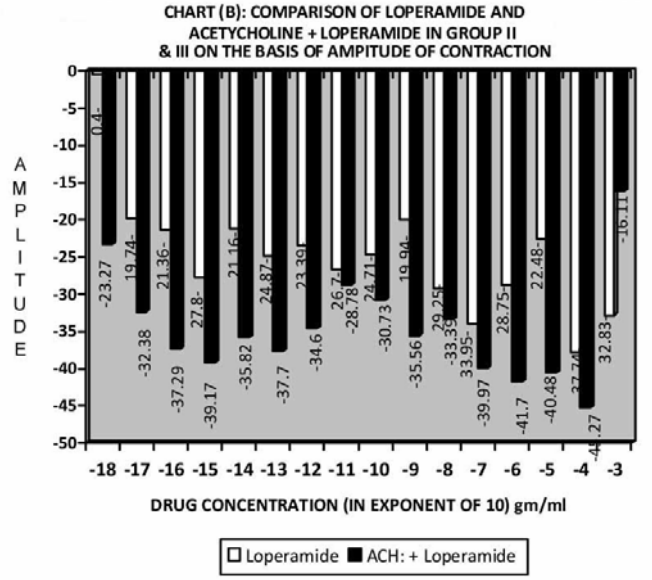
PURPOSE OF STUDY:

To study the inhibitory effects of loperamide on spontaneously contracting ileum and compare it with inhibitory effects of loperamide on acetylcholine induced contractions of isolated Guinea pig ileum.

MATERIALS AND METHODS:

All experimental work was carried out in the Department of Pharmacology and Therapeutics, Basic Medical Science Institute (BMSI), Jinnah Post Graduate Medical Center (JPMC) Karachi. Loperamide salt was supplied by ZAFA Pharmaceuticals Pakistan (Ltd) Karachi, while Acetylcholine salt was supplied from Department of Pharmacology & Therapeutics, Basic Medical Science Institute (BMSI), and Jinnah Postgraduate Medical Center (JPMC) Karachi. Serial dilutions of loperamide and acetylcholine were made from 10^{-3} to 10^{-18} gm/ml. The aerated (oxygenated) and fresh specified Tyrode physiological nutrient solution (contains Sodium Chloride, Potassium Chloride, Calcium Chloride Anhydrous, Magnesium Chloride, Sodium Bicarbonate, Sodium Dihydro Phosphate, Dextrose, and Distilled Water) was used for the perfusion of isolated intestinal segments. Twenty four healthy adult guinea pigs of both sexes (non pregnant) obtained from animal house of J P M C Karachi, weighing 450 – 750 gm were used. The animals were sacrificed by a blow on the head with hammer (approval for animal experimentation[26]). The abdomen was opened and ileum was located. A piece of terminal ileum was separated and transferred to Petri dish containing aerated (oxygenated) Tyrode solution. A segment of about 15 – 20 mm long was taken from isolated ileum, and mounted vertically in inner organ bath (which contains 20 ml aerated Tyrode solution). It was connected to 7B Grass Polygraph machine with the help of force displacement transducer, and was subjected to the tension of 1 gm. Temperature of organ bath was maintained at 37°C. Preparations were allowed to stabilize 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.





Study samples were divided in three groups and eight experiments were performed in each group. In group-I, the tissues were exposed to serial dilutions of acetylcholine (from10^{-18} to 10^{-3} gm/ml) and standard (control) concentration of acetylcholine was selected (10^{-6}gm/ml), that had produced maximum stimulation. In group-II, the tissues were exposed to serial dilutions (from10^{-18} to 10^{-3} gm/ml) of loperamide. While in group III, initially the tissues were challenged with control concentration of acetylcholine (10^{-6}gm/ml), than same tissues were exposed to serial dilutions (from 10^{-18} to 10^{-3} gm/ml) of loperamide. Responses were recorded by 7B Grass Polygraph machine for two minutes for each dilution in each group.

RESULTS:

Results were based on the frequency and amplitude of contractions. In group II, decreased the frequency of contraction ranging from 13.17% to 25.36% as the concentration of loperamide increased, but inhibitory response of loperamide at its higher concentration (10^{-3} gm/ml) was decreased (Chart A). The amplitude of contraction also decreased ranging from 19.74% to 37.74% as the concentration

Table (A) comparison of Lopramide and Loperamide + acetylcholine in Group II and III on the basis of frequency (rate) of contraction

Drug	Concentration (gm/ml)	Baseline Frequency	Drug Frequency	Decrease (%)
		Mean ± S.E.M	Mean ± S.E.M	
Loperamide	10^{-18}	25.63 ± 1.99	22.25 ± 1.8	13.17
ACH+Lopera	10^{-18}	19.25 ± 2.74	14.38 ± 2.65	25.32
	p - value	P < 0.05	P < 0.02	
Loperamide	10^{-17}	25.63 ± 1.40	21.75 ± 1.6	15.14
ACH+Lopera	10^{-17}	19.75 ± 2.19	13.75 ± 2.43	30.38
	p - value	P < 0.02	P < 0.02	
Loperamide	10^{-16}	26.50 ± 1.30	22.13 ± 1.1	16.51
ACH+Lopera	10^{-16}	24.88 ± 3.23	15.88 ± 2.36	27.43
	p - value	P < 0.05	P < 0.02	
Loperamide	10^{-15}	26.50 ± 2.33	21.13 ± 2.1	20.28
ACH+Lopera	10^{-15}	24.13 ± 2.26	16.88 ± 2.66	30.05
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-14}	25.00 ± 1.71	19.63 ± 1.8	21.50
ACH+Lopera	10^{-14}	25.25 ± 3.03	19.63 ± 2.76	22.28
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-13}	25.88 ± 2.32	20.88 ± 2.1	19.32
ACH+Lopera	10^{-13}	23.88 ± 2.75	18,38 ± 2.58	23.04
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-12}	25.88 ± 2.63	20.75 ± 2.2	19.81
ACH+Lopera	10^{-12}	23.63 ± 3.14	17.13 ± 3.26	27.51
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-11}	26.00 ± 2.64	20.50 ± 1.9	21.15
ACH+Lopera	10^{-11}	24.13 ± 2.52	16.50 ± 2.50	31.16
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-10}	26.63 ± 2.45	20.88 ± 2.2	21.60
ACH+Lopera	10^{-10}	23.00 ± 2.61	16.00 ± 2.31	30.43
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-9}	26.75 ± 2.22	21.88 ± 1.9	18.22
ACH+Lopera	10^{-9}	22.50 ± 2.47	14.88 ± 2.07	33.89
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-8}	27.50 ± 2.41	21.25 ± 1.8	22.73
ACH+Lopera	10^{-8}	22.38 ± 2.24	13.75 ± 2.40	38.55
	p - value	P < 0.02	P < 0.02	
Loperamide	10^{-7}	26.25 ± 2.72	21.50 ± 1.6	18.10
ACH+Lopera	10^{-7}	22.88 ± 2.47	13.75 ± 2.73	39.89
	p - value	P < 0.02	P < 0.02	
Loperamide	10^{-6}	25.13 ± 2.07	20.13 ± 1.8	19.90
ACH+Lopera	10^{-6}	22.75 ± 2.24	15.00 ± 2.54	34.07
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-5}	26.25 ± 2.16	19.75 ± 2.1	24.76
ACH+Lopera	10^{-5}	24.50 ± 1.61	16.25 ± 2.38	33.67
	p - value	N.S	N.S.	
Loperamide	10^{-4}	26.13 ± 2.50	19.50 ± 2.2	25.36
ACH+Lopera	10^{-4}	23.88 ± 1.86	17.88 ± 2.47	25.13
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-3}	28.00 ± 1.98	21.75 ± 1.5	22.32
ACH+Lopera	10^{-3}	24.38 ± 2.03	19.00 ± 2.23	22.05
	p - value	P < 0.05	P < 0.05	

Table (B) comparison of Lopramide and Loperamide + acetylcholine in Group II and III on the basis of amplitude of contraction.

Drug	Concentration (gm/ml)	Baseline Amplitude (mm)	Drug Amplitude (mm)	Decrease (%)
		Mean ± S.E.M	Mean ± S.E.M	
Loperamide	10^{-18}	94.00 ± 6.54	93.63 ± 15.13	0.40
ACH+Lopera	10^{-18}	184.25 ± 29.11	141.38 ± 22.78	23.27
	p - value	P < 0.005	P < 0.001	
Loperamide	10^{-17}	136.75 ± 5.57	109.75 ± 8.30	19.74
ACH+Lopera	10^{-17}	157.13 ± 31.30	106.25 ± 18.62	32.38
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-16}	152.00 ± 14.39	120.13 ± 16.96	21.36
ACH+Lopera	10^{-16}	188.25 ± 41.99	136.88 ± 32.99	27.29
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-15}	152.00 ± 21.32	109.75 ± 15.18	27.80
ACH+Lopera	10^{-15}	242.88 ± 71.96	147.75 ± 37.10	39.17
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-14}	152.38 ± 22.91	120.13 ± 21.20	21.16
ACH+Lopera	10^{-14}	252.63 ± 60.29	162.13 ± 35.15	35.82
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-13}	172.88 ± 18.97	129.88 ± 18.10	24.87
ACH+Lopera	10^{-13}	274.50 ± 56.70	171.00 ± 38.28	37.70
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-12}	172.25 ± 19.27	132.13 ± 18.95	23.29
ACH+Lopera	10^{-12}	295.50 ± 59.21	193.25 ± 38.02	34.60
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-11}	174.63 ± 19.65	128.00 ± 16.43	26.70
ACH+Lopera	10^{-11}	277.13 ± 46.02	197.38 ± 41.90	28.78
	p - value	P < 0.02	P < 0.05	
Loperamide	10^{-10}	183.63 ± 17.84	138.25 ± 18.65	24.71
ACH+Lopera	10^{-10}	296.13 ± 47.82	205.13 ± 36.62	30.73
	p - value	N P < 0.001	P < 0.05	
Loperamide	10^{-9}	181.75 ± 11.54	145.50 ± 10.95	19.94
ACH+Lopera	10^{-9}	318.50 ± 53.06	205.25 ± 28.68	35.56
	p - value	P < 0.02	P < 0.05	
Loperamide	10^{-8}	155.13 ± 14.08	109.75 ± 12.86	29.25
ACH+Lopera	10^{-8}	341.38 ± 60.54	227.38 ± 41.36	33.39
	p - value	P < 0.01	p<0.01	
Loperamide	10^{-7}	176.00 ± 19.78	116.25 ± 11.50	33.95
ACH+Lopera	10^{-7}	291.50 ± 57.00	175.00 ± 40.60	39.97
	p - value	P < 0.05	P < 0.05	
Loperamide	10^{-6}	173.50 ± 12.40	123.63 ± 12.56	28.75
ACH+Lopera	10^{-6}	331.50 ± 59.28	193.25 ± 39.87	41.70
	p - value	P < 0.02	P < 0.05	
Loperamide	10^{-5}	147.38 ± 14.32	114.25 ± 12.57	22.48
ACH+Lopera	10^{-5}	326.38 ± 55.88	194.25 ± 44.82	40.48
	p - value	P < 0.01	P < 0.05	
Loperamide	10^{-4}	167.25 ± 17.47	104.13 ± 13.55	37.74
ACH+Lopera	10^{-4}	326.38 ± 55.88	178.25 ± 49.07	45.27
	p - value	P < 0.02	P < 0.05	
Loperamide	10^{-3}	147.38 ± 14.65	99.00 ± 9.65	32.83
ACH+Lopera	10^{-3}	251.38 ± 83.63	210.88 ± 80.91	16.11
	p - value	P < 0.05	P < 0.05	

of loperamide increased, but inhibitory response of loperamide at its higher concentration (10^{-3} gm/ml) was decreased (Chart B).
In group III, loperamide decreased the frequency of contraction ranging from 25.32% to 39.89% as the concentration of loperamide increased, but inhibitory response of loperamide at its higher concentration (10^{-3} gm/ml) was decreased (Chart A). The amplitude of contraction also decreased ranging from 32.38% to 45.27% as the concentration of loperamide increased, but inhibitory response of loperamide at its higher concentration (10^{-3} gm/ml) was decreased (Chart B).
Statistically: We observed that at 10^{-9} to 10^{-6} gm/ml concentrations of loperamide, there was significant ($p<0.05$) difference in inhibitory responses of loperamide (on both frequencies and amplitudes of contractions) in group II and III, with higher inhibitory values in group III.

DISCUSSION:

The main object of this study was to investigate the hypothesis that the loperamide inhibits the contractions of primed Guinea pig ileum more in comparison to spontaneous contractions of Guinea pig ileum. Taqvi and et all (2009) observed the inhibitory response of loperamide on spontaneous contractions of isolated jejunum of rabbit at concentration of 1-10 M[15]. The results of Taqvi's study supports our study that loperamide inhibit spontaneous contractions significantly ($p<0.05$) at 10^{-4} gm/ml. Kojima R (2009) observed in his study that loperamide prolonged the evacuation time of intestine in mice (i.e. three times more the evacuation time compared to control)[16]. We also observed in vitro that loperamide decreases the contraction of small intestine (i.e. frequency for 25.5% and amplitude for 37.74%). Unar M.A (2010) observed in his study that loperamide and verapamil were found potent inhibitors of acetylcholine induced contractions of isolated guinea pig ileum. Our observations about loperamide also match that study[22]. Daly J.W and Harper J (2000) say that lopermide is widely used antidiarrheal agent that primarily acts at nano molar concentration through activation of opioid receptors in the gastrointestinal tract. At some what higher concentrations loperamide blocks (calmodulin activity) calcium channels[17]. Our observations were in complete agreement with these responses of loperamide that at lower concentration {froim10^{-3} to 10^{-9}gm/ml} loperamide decreases the frequency and amplitude non – significantly, where as at some what higher concentration (from 10^{-8} gm /ml to 10^{-4} gm /ml) loperamide significantly ($p<0.01$) decreased the frequency as well as amplitude of spontaneous as well as of acetylcholine induced contractions of isolated ileum of Guinea pig. While at higher (10^{-3} gm/ml) concentration loperamide showed decrease in its inhibitory response on frequency and amplitude of spontaneous as well as of acetylcholine induced contractions of isolated ileum of Guinea pig.

CONCLUSION

After statistical analysis and percentage wise comparison of inhibitory effects of loperamide in groups II and III, we observed that loperamide had more inhibitory effects on acetylcholine stimulated contractions than on spontaneously contractions of isolated Guinea pig ileum. While at higher concentrations (10^{-3} gm/ml) loperamide's inhibitory effects were decreased, perhaps due to blockage of calcium influx by loperamide. "However, lot of work is still required to establish the inhibitory effects of loperamide on other parts of gastrointestinal tract and also on other contractile cells of body.

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