Original Article

Reversal of Loperamide Induced Intestinal Smooth Muscle Relaxation by Glibenclamide and Repaglinide in Vitro

Effects of Glibenclamide and Repaglinide

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ABSTRACT

Objective: To compare the inhibitory effects of Glibenclamide and Repaglinide on loperamide induced relaxation of isolated ileum of Rabbit.

Study Design: Comparative controlled in-vitro experimental Study.

Place and Duration of Study: This study was conducted at Department of Pharmacology, Yusra Medical & Dental College Islamabad from February to April 2014.

Materials and Methods: Isolated pieces of small intestine of rabbits placed in freshly prepared Tyrode nutritional solution. Six groups were designed. In group I, effect of Acetylcholine on the intestine was observed. In group II ileum was exposed to serial dilutions of acetyl choline in the presence of fixed concentration of loperamide 10^{-6}, dose response curve was plotted. In group III fixed dose of Glibenclamide 10^{-6} was given and dose response curve was plotted with Acetylcholine. In group IV fixed dose of Repaglinide 10^{-6} was given and dose response curve was plotted with Acetylcholine. Group V was given Loperamide+Glibenclamide and dose responce curve was plotted with Acetylcholine, while group VI was given Loperamide+Repaglinide and dose response curve was plotted with Acetylcholine. The effects were observed and recorded on Power lab .

Results: Acetyl choline has produced dose dependent increase in force of contraction from 4.9 to 7.2 mN. In the presence of glibenclamide the force of intestinal smooth muscle contraction increase from 6.4 to 7.8mN and in the presence of loperamide the force decreased from 4.8 to 3.03mN. In the end effect observed with acetyl choline in the presence of loperamide and glibenclamide is 6.5 to 7.7mN. Similarly with repaglinide alone the force of contraction increased from 5.4 to 9.6mN and with repaglindie + loperamide from 4.3 to 21.5 mN. On statistical analysis 't' test was applied and P value was found to be significant that is $P<0.05$.

The dose response curve of acetylcholine on intestinal smooth muscle of rabbit shifted towards left side with glibenclamide and rapaglinide alone. In the presence of Loperamide the curve shifted towards right side. Glibenclamide and repaglinide when given together with loperamide respectively lead to leftwards shift of the dose response curve.

Conclusion: Hence sulfonylurea glibenclamide and repaglinide, the oral anti-diabetics effectively reversed the relaxation of intestinal smooth muscle by loperamide.

Key Words: Loperamide, Relaxation, Meglitinide, Repaglinide, K^+ ATP Channel

INTRODUCTION

Opiate-induced constipation (OIC) is widely observed among patients receiving chemotherapy[1]. In the gastrointestinal system, the opioid peptides are released and activate opioid receptors, which regulate the enteric circuitry by controlling motility and secretion. Together with the inhibition of ion and fluid secretion, these effects result in constipation, one of the most troublesome side effects of opiate analgesic treatment[2]. The development of a better therapy for treating OIC is urgent and necessary. Loperamide is widely used clinically to treat a variety of diarrheal syndromes, including acute and nonspecific (infectious) diarrhea[3]. Loperamide is a peripheral agonist of opioid μ-receptors with poor ability to penetrate the blood-brain barrier [3]. Opioid μ-receptors are divided into three subtypes: μ-1, μ-2 and μ-3 . The activation of opioid μ-1 receptors has been reported to be associated primarily with the phospholipase C (PLC)-protein kinase C (PKC) pathway[4]. PLC-PKC signals can increase the intracellular calcium concentration, inducing gastrointestinal or bladder contraction [5,] Therefore, it is unlikely that intestinal relaxation is induced by the activation of opioid μ-1 receptors. ATP-sensitive K^+ (K_{ATP}) channels are involved in the regulation of intestinal smooth muscle.[6] In addition, the opening of K_{ATP} channels has been reported to reduce intracellular Ca^+ concentration[7]. The K_{ATP} channel opener diazoxide has been shown to have the ability to attenuate indomethacin-induced small intestinal damage in rats [8]. However, the role of K_{ATP} channels in loperamide-induced gastrointestinal transit remains obscure.

Glibenclamide a second generation sulphonylurea, inhibits an ATP-dependent K+ (KATP) channel on the cell membrane of pancreatic beta cells. This depolarization opens voltage-gated Ca2+ channels. The

rise in intracellular calcium leads to increased release of insulin[9] .

Repaglinide belongs to meglitinide class and is used in the management of type 2 diabetes mellitus. This depolarizes the beta cells, opening the cells' calcium channels, and the resulting calcium influx induces insulin secretion[10]

Acetylcholine acts in the gut by stimulation of M_3 muscarinic receptors subtypes, and causes increased contractions of the small intestine[11,12].

In the present study an attempt has been made to cause reversal of loperamide induced relaxation by glibenclamide and rapaglinide as they block K^+ channel.

MATERIALS AND METHODS

All experimental work was carried out in the Department of Pharmacology and Therapeutics, Yusra Medical & Dental College Islamabad. Loperamide Hydrochloride, Acetylcholine, Glibenclamide and Repaglinide was supplied by medizan laboratories (pvt) ltd Pakistan. Serial dilutions of loperamide and acetylcholine were made from 10^{-3} to 10^{-9} gm/ml. The aerated (oxygenated) and fresh specified Tyrode physiological nutrient solution was used for the perfusion of isolated intestinal segments. Healthy rabbits of both sexes (non pregnant) obtained from animal house of college. All animal-handling procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the Guidelines of the Animal Welfare Act.

The animals were slaughtered (approval for animal experimentation 26). 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 the force transducer. 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 Six groups and six 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 of acetylcholine in the presence of fixed concentration $10^{-6}$of glibenclamide.. While in group III, the tissues were exposed to serial dilutions of acetyl choline in the presence of fixed concentration $10^{-6}$of loperamide [19] In group IV the tissues were exposed to serial dilutions of acetylcholine in the presence of fixed concentration $10^{-6}$of Rapaglinide. In group V effect of serial dilutions of acetylcholine were recorded in presence of loperamide + glibenclamide. In group VI effect of serial dilutions of acetylcholine were recorded in presence of loperamide and rapaglinide. Responses were recorded on Power Lab machine for 30 sec or each dilution in each group

Statistical Analysis: Analysis was done on SPSS version 14 and 't' test was used to evaluate the significance between groups. $P<0.05$ was considered to be a significant

RESULTS

Effect of increasing concentration of acetylcholine in the presence of Glibenclamide on intestinal smooth muscle: Intestinal strips were exposed to serial dilutions of acetyl choline from 10^{-8} to 10^{-6} M. and the force of contraction increased from 6.4 to 7.8 mN as shown in Table 1

Table No.1: Effect of increasing concentration of acetylcholine in the presence of Glibenclamide on intestinal smooth muscle

Sr. No.	Dose µg (Ach)	Conc. (M)	log dose conc.	Force of Contraction (mN)	%age Response
1	0.01	-8	-2.00	6.4	82.05
2	0.05	-8	-1.30	7	89.74
3	0.1	-7	-1.00	7.2	92.31
4	0.5	-7	-0.30	7.5	96.15
5	1	-6	0.00	7.7	98.72
6	5	-6	0.70	7.8	100

Effect of increasing concentration of acetylcholine in the presence of loperamide on intestinal smooth muscle: Intestinal strips were exposed to serial dilutions of acetyl choline from 10^{-8} to 10^{-6} M. in the presence of loperamide and the force of contraction decreased from 4.85 to 3.85 mN as shown in Table 2

Table No.2: Effect of increasing concentration of acetylcholine in the presence of loperamide on intestinal smooth muscle.

Sr. No.	Dose µg (Ach)	Conc. (M)	log dose conc.	Force of Contraction (mN)	%age Response
1	0.01	-8	-2.00	4.85	160.07
2	0.05	-8	-1.30	4.34	143.23
3	0.1	-7	-1.00	4.04	133.33
4	0.5	-7	-0.30	3.85	127.06
5	1	-6	0.00	3.18	104.95
6	5	-6	0.70	3.03	100

Effect of increasing concentration of acetylcholine in the presence of loperamide + Glibenclamide on intestinal smooth muscle: Intestinal strips were exposed to serial dilutions of acetyl choline from 10^{-8} to 10^{-6} M. in the presence of loperamide and Glibenclamide and the force of contraction increased from 6.5 to 7.7 mN as shown in Table 3.

Dose Response Curves with the 4 groups of drugs: The dose response curves of the mean values of all

groups are plotted and are shown in figure I On comparison between groups the curve with acetylcholine and loperamide is reversed in the presence of glibenclamide. On applying 't' test P values are found to be significant as shown in Table 4 & 5.

Table No.3: Effect of increasing concentration of acetylcholine in the presence of loperamide + Glibenclamide on intestinal smooth muscle.

Sr. No	Dose µg (Ach)	Conc. (M)	log dose conc.	Force of Contraction (mN)	%age Response
1	0.01	-8	-2.00	6.5	84.42
2	0.05	-8	-1.30	6.7	87.01
3	0.1	-7	-1.00	7	90.91
4	0.5	-7	-0.30	7.2	93.51
5	1	-6	0.00	7.6	98.70
6	5	-6	0.70	7.7	100

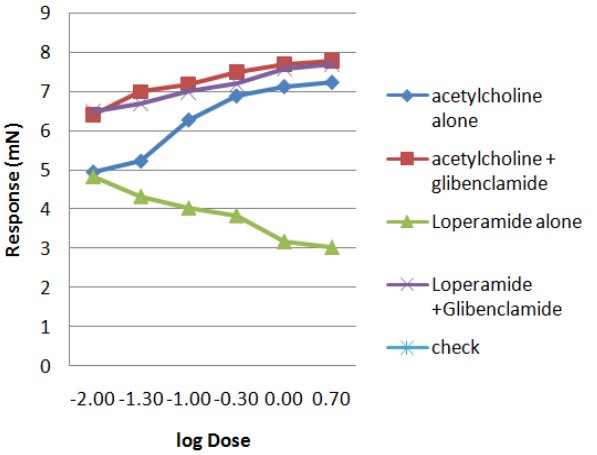

Figure No.1: Dose response curve of acetyl choline in presence of glibenclamide, loperamide + glibenclamide

Table No.4: Comparison between groups also showing P values

Sr. No	Dose of acetylc Acetylcholine in µg	Conc. (M)	Effect of acetylcholine mN (Ach)	Force of Contraction (mN) in the presence of glibenclamide alone	Force of Contraction (mN) in the presence of loperamide	Force of Contraction (mN) in the presence of glibenclamide + loperamide
1	0.01	-8	4.94	6.4	4.85	6.5
2	0.05	-8	5.22	7	4.34	6.7
3	0.1	-7	6.27	7.2	4.04	7
4	0.5	-7	6.88	7.5	3.85	7.2
5	1	-6	7.12	7.7	3.18	7.6
6	5	-6	7.23	7.8	3.03	7.7
P value				0.002664	0.008227 (Ach) 0.00049 (glibenclamide)	0.006407 (Ach) 0.029958 (glibenclamide) 0.000531 (loperamide)

Table No.5: Effect of increasing concentration of acetylcholine in the presence of Repaglinide on intestinal smooth muscle

Sr. No	Dose µg (Ach)	Conc. (M)	log dose conc.	Force of Contraction (mN) (Repaglinide)	%age Response
1	0.01	-8	-2.00	5.44	56.67
2	0.05	-8	-1.30	7.33	76.35
3	0.1	-7	-1.00	7.71	80.31
4	0.5	-7	-0.30	7.94	82.71
5	1	-6	0.00	8.12	84.58
6	5	-6	0.70	9.6	100.00

Effect of increasing concentration of acetylcholine in the presence of loperamide on intestinal smooth muscle: Intestinal strips were exposed to serial dilutions of acetyl choline from 10^{-8} to 10^{-6} M. and the force of contraction decreased from 4.8 to 3.0 mN as shown in Table 6.

Effect of increasing concentration of acetylcholine in the presence of Rapaglinide + loperamide on intestinal smooth muscle: Intestinal strips were exposed to serial dilutions of acetyl choline from 10^{-8} to 10^{-6} M. and the force of contraction increased from 4.37 to 21.5 mN

Table No.6: Effect of increasing concentration of acetylcholine in the presence of loperamide on intestinal smooth muscle

Sr. No	Dose µg (Ach)	Conc. (M)	log dose conc.	Force of Contraction (mN) (loperamide)	%age Response
1	0.01	-8	-2.00	4.85	160.07
2	0.05	-8	-1.30	4.34	143.23
3	0.1	-7	-1.00	4.04	133.33
4	0.5	-7	-0.30	3.85	127.06
5	1	-6	0.00	3.18	104.95
6	5	-6	0.70	3.03	100

Dose Response Curves with the 4 groups of drugs: The dose response curves of the mean values of all groups are plotted and are shown in figure II On comparison between groups the curve with acetylcholine and loperamide is reversed in the presence of Repaglinide.

In figure III bar diagram show the comparison between drugs acetylcholine alone, acetylcholine in the presence of loperamide, Repaglinide and loperamide + Repaglinide.

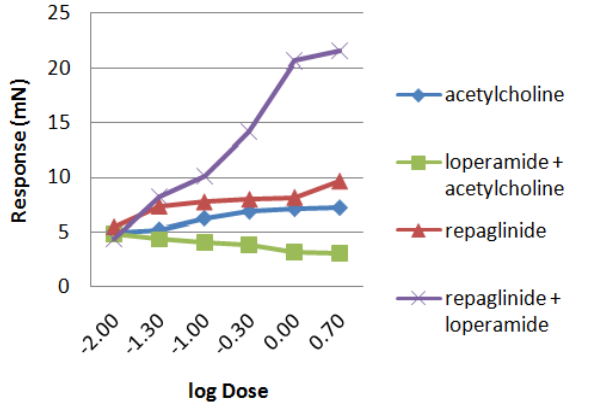

Figure No.2: Dose-Response curves with acetylcholine alone and in the presence of loperamide, repaglinide and repaglinide + loperamide.

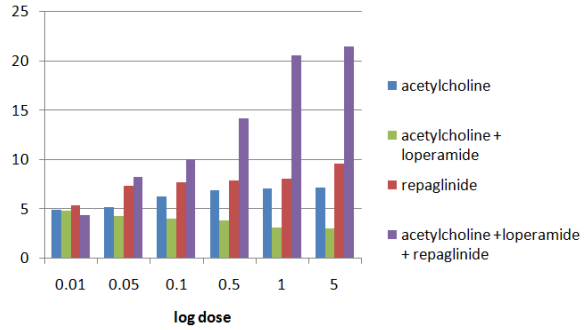

Figure No.3: Bar diagram showing comparison of effects between effects of acetylcholine, Repaglinide, loperamide and Repaglinide + loperamide.

DISCUSSION

In this study it has been seen that the dose response curve of acetylcholine on intestinal smooth muscle of rabbit is shifted toward left side with glibenclamide and rapaglinide alone. In the presence of Loperamide the curve is shifted toward right side. Glibenclamide and repaglinide when given together with loperamide respectively lead to leftward shift of the dose response curve.

The action of loperamide that it causes relaxation of intestinal smooth is related to the activation of opioid receptors in peripheral tissues.[13] Loperamide exert this action by stimulation of μ-2 opioid receptors.[14,15] These receptors lead to activation of K_{ATP} channels which causes hyper polarization of smooth muscle membrane and then its relaxation.

Sulfonylureas (glibenclamide and repaglinide) stimulate insulin secretion from pancreatic β-cells and are widely used to treat type 2 diabetes. Their principal target is the ATP-sensitive potassium (K_{ATP}) channel, which plays a major role in controlling the β-cell membrane potential. Inhibition of K_{ATP} channels by glucose or sulfonylureas causes depolarization of the β-cell membrane; in turn, this triggers the opening of voltage-gated Ca^{2+} channels, eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+} which stimulates the exocytosis of insulin-containing secretory granules[16].

The K^+ ATP channel is a hetero-octameric complex of two different types of protein subunits an inwardly rectifying K^+ channel, Kir6.x, and a sulfonylurea receptor, SUR[23]. Sulfonylureas (e.g., tolbutamide, gliclazide, glimepiride and benzamido derivatives (e.g meglitinide) close K_{ATP} channel by binding with high affinity to SUR [17]

Hence in comparison to study conducted by Chih-Cheng lu it was seen that loperamide induced relaxation of prostatic strip was abolished by pre-treatment with glibenclamide[18]. We have found similar results on intestinal smooth muscle of rabbit. In this study Rapaglinide also has reversed the loperamide induced relaxation in similar way as glibenclamide. Rapaglinide is the member of meglitinide group of insulin secretagogues and act in a similar way as sulfonylureas. The binding sites of rapaglinide on K^+ ATP channel is similar as sulfonylurea and also has one unique binding site. [19]

Loperamide causes relaxation of the intestinal smooth muscle through myenteric plexus, which is the basic mechanism through which it causes its anti diarrheal effect. However, in case of long term use of loperamide toxic megacolon and paralytic ileus have been reported. Since according to this study the smooth muscle relaxation induced by loperamide can be reversed by the use of Glibenclamide, these drugs can prove to be useful in order to prevent or reverse toxic megacolon and ileus, induced by the long term use of loperamide

With the increased use of opioids, there are more patients presenting with Opiate induced constipation or opiate bowel dysfunction (OBD).[20] Constipation may be debilitating among those who require chronic analgesia [21]; OIC or OBD affected an average of 41 % patients taking an oral opioid for up to 8 weeks in a meta-analysis of 11 placebo-controlled, randomized studies in non-malignant pain[22]. Patients may discontinue treatment due to constipation, despite their established need for long-term pain relief. For treatment of this naloxone is used. The therapeutic index of naloxone is very narrow. So, Sulfonylureas along with glucose can be a good substitute for treatment of opioid induced constipation as seen in this study.

CONCLUSION

In conclusion, we suggest that activation of opioid μ-2 receptors to open K_{ATP} channels is responsible for loperamide-induced intestinal relaxation which is blocked by glibenclamide and rapaglinide as they are k^+ chaanel blockers in pancreatic beta cells.

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