Original article

Comparison of the Effect of Dipeptidyl Peptidase-4 Inhibitors Sitagliptin and Vildagliptin on Intestinal Motility of Rabbit Ileum and Rat Gastric Fundus Tissue

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ABSTRACT

Background: Diabetes mellitus is a pervasive metabolic disorder. Its prevalence is very high in developing as well as in developed countries. Long-standing disease may result in many microvascular and macrovascular complications including diabetic gastroparesis which adversely affects the quality of life of patients. Research to evaluate the impact of antidiabetic drugs on intestinal motility and to evaluate their possible drug interactions is an important field of research these days. The objective of this study was to evaluate the effect of sitagliptin and vildagliptin on intestinal motility of rabbit ileum and rat gastric fundus tissue alone and in the presence of ondansetron and to compare their effect.

Materials and methods: This Experimental study was performed in CMH Lahore Medical College and FJMU, Lahore, on isolated tissues obtained from adult healthy rabbits and rats over 18 months. Animals were divided into four groups of each species; each group comprised twenty animals. Strips of rabbit ileum and rat gastric fundus tissues were allowed to stand in physiological salt solution in an organ bath with transducers attached to the Powerlab. Increasing concentrations of Sitagliptin and Vildagliptin were administered and a change in intestinal motility was recorded by powerlab (AD instruments) as a change in the force of contraction(g). Then increasing concentrations of sitagliptin and vildagliptin,were administered in the presence of ondansetron. Unpaired t-test was used to compare the difference between the two groups of drugs A p-value of less than 0.05 was considered significant.

Results: There was a dose-dependent inhibitory effect of sitagliptin and vildagliptin on intestinal motility.Sitagliptin has an inhibitory effect when administered alone and change the force of contraction from 19.86±1.71 to 14.00±1.5 on rabbit ileum and from 9.008±1.3 to 6.4±1.18 on rat gastric fundus tissue. Sitagliptin in the presence of ondansetron has dual effect at low concentrations it has inhibitory effect and at high concentration it has stimulatory effect. . Sitagliptin has more significant inhibitory effects on the force of contraction as compared to vildagliptin (p-value < 0.001).

Conclusion: The Present study confirms the inhibitory effect of sitagliptin and vildagliptin on the gastrointestinal motility of rabbit ileum and rat gastric fundus tissue.

Keywords:

Sitagliptin, Vildagliptin, Gastroparesis, Isolated tissue

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from either a decreased production of insulin from pancreatic beta cells (type_1) or the presence of insulin resistance (type-II)[1]. The number of patients suffering from DM has increased enormously in the last three decades. According to the World Health Organization (WHO), diabetes will be the 7th major cause of death by the year 2030[2]. The International Diabetes Federation (IDF) has expressed that almost 75% of patients with DM are residing in underdeveloped countries, whereas 81.2% of people are undiagnosed across the globe. Also, people suffering from DM are young, aged 35-64 years in developing countries as compared to developed countries where people suffer from diabetes in old age. WHO states that in underdeveloped countries, the number of people with diabetes will further increase by 150% in the next two and half decades.[3] The rate of diabetes mellitus is also very high in Pakistan. Around 8 million people are suffering from diabetes. And Pakistan is the fourth largest country on the list, which will double by 2025, according to WHO[4].

Major causative factors for diabetes mellitus include obesity, physical inactivity, high BMI, and genetic factors resulting in symptoms like polyuria, polyphagia, and polydipsia. Persistent hyperglycaemia can lead to various complications which are classified as microvascular and macrovascular complications.[5] One of the most common complications is diabetic

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gastroparesis characterized by early satiety, abdominal distention, and postprandial fullness, resulting in nausea and vomiting, abdominal pain, and constipation or diarrhea[6]. Persistent hyperglycaemia creates an environment of inflammation, oxidative stress, and deficiency in levels of important neurotransmitters like nerve growth factors, serotonin, and local hormones[7]. Also, gut microbiota plays a role by further decreasing the production of short-chain fatty acids which enhance the secretion of the incretin hormones. Reduced levels of incretin hormones not only influence glucose metabolism but also increase low-grade inflammation[8].

There are many treatment strategies available for the treatment of DM such as lifestyle modifications, oral hypoglycaemic agents, insulin therapy, and gene therapies. Most of these treatment strategies are either expensive or have many adverse effects such as hypoglycemia, hypothyroidism, atherosclerosis abdominal pains, and insulin resistance[9]. Hence management of diabetes mellitus and its complications is still a worldwide problem[10].

DPP-4 inhibitors such as vildagliptin, sitagliptin, Alogliptin, etc. are one of the commonly used antihyperglycemic agents these days. The mechanism of action of DPP-4 inhibitors is based on inhibition of the metabolism of various gut incretin hormones such as glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide, and other glucose-dependent insulin-like peptides. Hence, they decrease fasting as well as post-prandial blood glucose levels. These hormones have a significant role in beta cell survival and increase beta cell mass and insulin production[11]. They reduce the inflammation in the gut wall. It is claimed that DPP-4 inhibitors also increase serum serotonin levels as evidenced by in vivo studies[12-13].

There is very little research showing the in vitro effect of DPP-4 inhibitors on intestinal motility which can further guide about their use in patients suffering from diabetic gastroenteropathy. Keeping all these factors in view this study was designed to evaluate the effect of DPP-4 inhibitors (Sitagliptin, Vildagliptin) on intestinal motility in isolated tissues such as rabbit ileum and rat gastric fundus tissue. Their effects were compared with each other and any drug interaction with ondansetron was observed.

MATERIALS AND METHODS

This experimental study was performed in CMH Lahore Medical College over a period of 18 months. Adult healthy rabbits (weighing 1-1.5 kg of either gender, non-pregnant) and Albino rats (150-200g, either gender, non-pregnant) were included in the study. Animals were divided into 4 groups comprising of 20 animals in each group. Following drugs were used in this research project. Ondansetron hydrochloride dihydrate (Indus Pharma), Vildagliptin (Novartis), Sitagliptin phosphate (Hilton Pharma). These drugs were obtained as a gift sample.

The experimental protocol was performed after permission from ethics review committee of FJMU, Lahore. All animals were purchased from the local market and animal house of CMH Lahore Medical College. They were placed in the animal house of CMH Lahore Medical College, under optimum hygienic conditions, natural day, and light cycle at normal room temperature. Animals were acclimatized for 1 week before commencement of the study. They had free access to food and water. Before the experiment animals were kept fasting for 18 hours while water was provided. All animals were handled according to the criteria explained in the Guide for Care and Use for Laboratory Animals[14]. Molar concentrations of solutions were prepared according to the molecular weights of drugs.

Preparation of Rabbit Ileum Tissue

Rabbits were euthanized by slaughtering. Animals were dissected and the ileum was removed and carefully separated from the mesentery. Ileum was put in Tyrode's solution. The composition of Tyrode's solution in mM was as follows: 11.90 $NaHCO_3$, 136.9 NaCl, 1.05 $MgCl_2$, 1.8 $CaCl_2$, 0.42 NaH_2PO_4 (pH 7.4), 2.68 KCl, and 5.55 glucose, 0.42 NaH_2PO_4. This solution was freshly prepared daily. Pieces of ileum having a length of 2 cm were suspended in organ baths (Radnoti 159920-X1/C; Radnoti Llc, Covina, CA) containing 25 ml of Tyrode's solution. The solution will be continuously aerated with carbogen (5% CO_2 and 95% O_2). After hanging the tissue, 1 g of resting tension was applied as preload. Before the addition of any drug, the tissue was allowed to equilibrate for almost 30 minutes. The changes in the tension were detected by using an isotonic transducer(model no MLT 0402) which was attached to the PowerLab data acquisition system (Model: PL26T04,AD Instruments, Sydney, Australia) and recorded on lab chart version 8 soft ware program (AD Instruments, Sydney, Australia) to measure the contractile force. [15]

Preparation of Rat Gastric Fundus Tissue

Rats were anesthetized by chloroform. The stomach was dissected out and separated from surrounding tissues. Strips having a width of 4 –5 mm and length of

almost 20 mm were prepared[16]. The tissue was mounted in a 25 mL organ bath. Kreb's solution has the composition (mM): $NaHCO_3$ 25.0, NaCl 118.2, $KH_2PO_4$1.3, $CaCl_2$ 2.5, KCl 4.7, $MgSO_4$ 1.2, and glucose 1.7 (pH 7.4) were used for experiment. The temperature of the tissue bath was maintained at 37 ± 1 °C and aerated continuously. A load of 1.0 g was applied and the responses were recorded after an equilibration period of 30 min.[17]Ccontractions in rat gastric fundus tissues were recorded by using an isotonic transducer(model no MLT 0402) which was attached to the PowerLab (Model: PL26T04) data acquisition system labchart version 8 (ADInstruments, Sydney, Australia).

The cumulative dose-response curves for sitagliptin and Vildagliptin alone were constructed. As the capacity of the organ bath was 25 ml, 25 µL of $1x10^{-6}$ molar solution was administered to get a final concentration of $1x10^{-9}$ or 0.001 micromoles in the organ bath. Then after getting maximum tissue response for up to ½ an hour.

The next concentration of 50 microliter 0f $1x10^{-6}$ was administered to get a final concentration of $3x10^{-9}$ or 0.003 µM in the organ bath. The same procedure was followed to administer increasing concentrations[18].

Cumulative dose-response curves of increasing concentrations of sitagliptin and vildagliptin were constructed after the pretreatment of tissue with ondansetron. For this purpose, tissue was initially exposed to 1µM of ondansetron solution for 30 minutes[19].

Statistical Analysis

All the Data was entered in the latest available version of Graph pad prism (version 8.01). The number of animals in each group were denoted as 'n'. Data was expressed as Mean ± SEM. T-test was used to compare the difference between the two groups whereas one-way ANOVA followed by Post Hoc Tukey's test was used for comparison among the large number of groups. A p-value of <0.05 was considered significant.

RESULTS

The cumulative dose-response curve for increasing concentrations of sitagliptin was plotted on rabbit ileum and rat gastric fundus tissue. There was an inhibitory effect on intestinal contraction as evidenced by a change in the mean force of contraction from 19.86±1.71 to 14.00±1.5 on rabbit ileum and from 9.008±1.3 to 6.4±1.18 on rat gastric fundus tissue.

The cumulative dose-response curve of increasing concentrations of sitagliptin was plotted in the presence of a fixed concentration of ondansetron on rabbit ileum and rat gastric fundus tissue . There was an inhibitory effect on lower concentrations up to 0.1 µM as shown by a decrease in the mean force of contraction from 13.52±3.62 to 13.16±3.64, a stimulatory effect was observed on intestinal motility as depicted by the change in the mean force of contraction from 13.32±3.66 to 14.86±3.82 on rabbit ileum. Similarly on rat gastric fundus tissue, there was a dose-dependent inhibition in response up to 0.3 micromole dose as shown by a change in the mean value of the force of contraction from 5.18 ±1.14 till 5.02 ±1.146 and then an increase in the mean force of contraction from 5.5±1.17 to 5.9±1.19.

An unpaired t-test was used to compare the means of the effects of sitagliptin alone and sitagliptin in the presence of a fixed concentration of ondansetron. P value < 0.0001 showed a significant difference between the two groups.

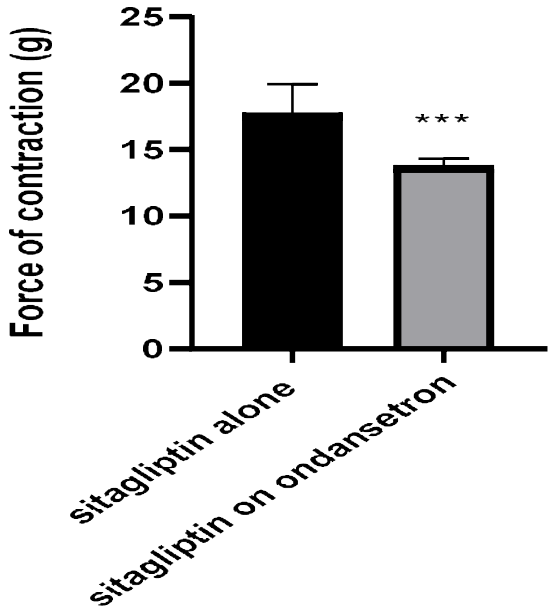

Fig 1: Comparison of effect of sitagliptin alone and sitagliptin in the presence of fixed concentration of ondansetron on rabbit ileum. (*** p-value <0.001, **p-value <0.01, *p-value <0.05)

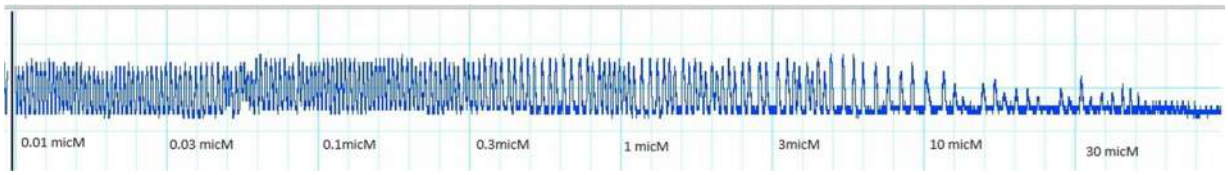

Fig 2: Tracing showing the effect of increasing concentrations of sitagliptin alone on rabbit ileum.

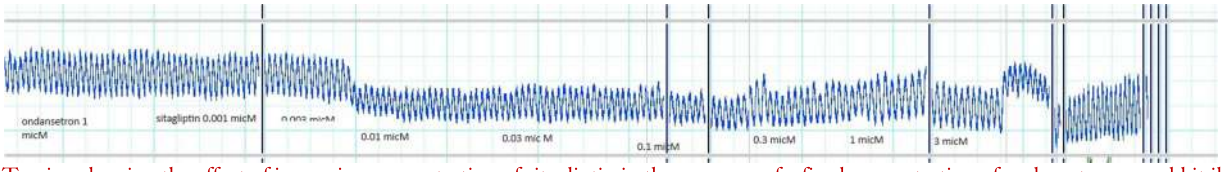

Fig 3: Tracing showing the effect of increasing concentration of sitagliptin in the presence of a fixed concentration of ondansetron on rabbit ileum.

Fig 4: Tracing showing the effect of increasing concentration of sitagliptin alone of rat gastric fundus tissue.

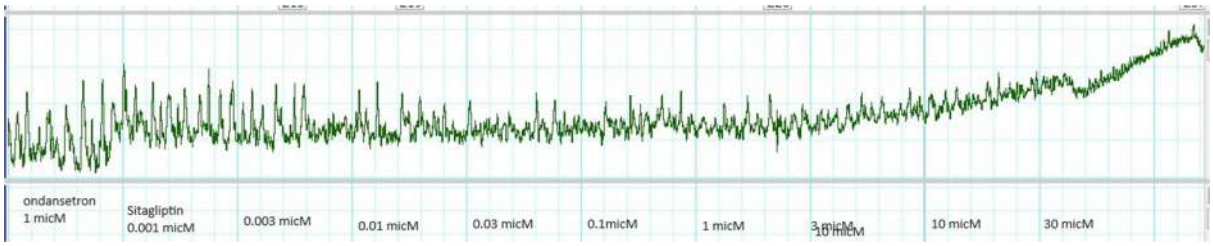

Fig 5: Tracing showing the effect of increasing concentration of sitagliptin in the presence of a fixed concentration of ondansetron on rat gastric fundus tissue.

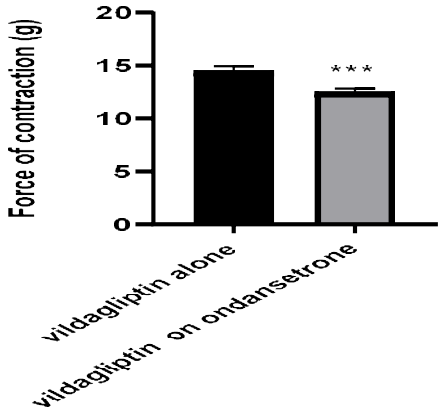

Fig 6: Comparison of the effect of increasing concentrations of vildagliptin alone and vildagliptin in the presence of a fixed concentration of ondansetron on rabbit ileum. (*** p-value < 0.001, **p-value <0.01, *p-value < 0.05)

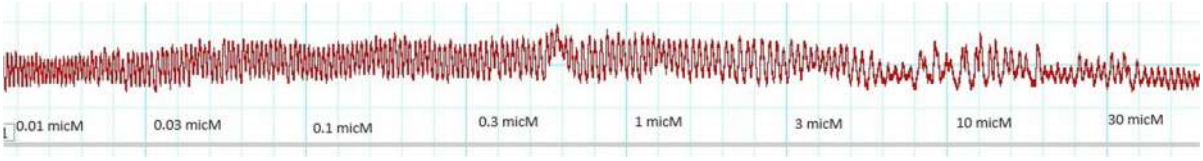

Fig 7: Tracing showing the effect of increasing concentration of vildagliptin alone on rabbit ileum

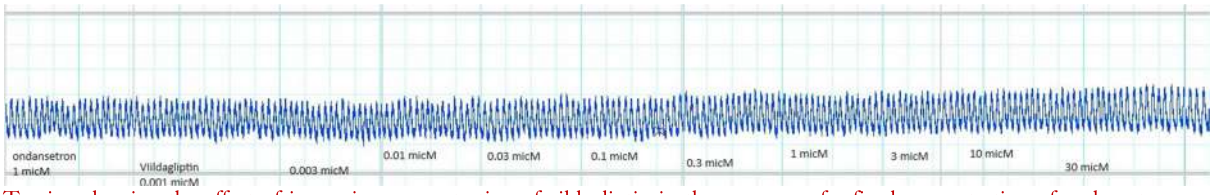

Fig 8: Tracing showing the effect of increasing concentration of vildagliptin in the presence of a fixed concentration of ondansetron on rabbit ileum.

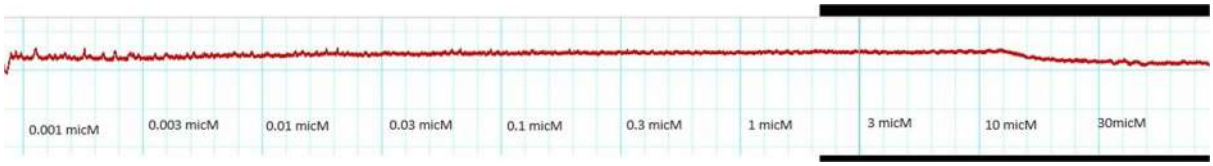

Fig 9: Tracing showing the effect of increasing concentration of vildagliptin alone on rat gastric fundus tissue.

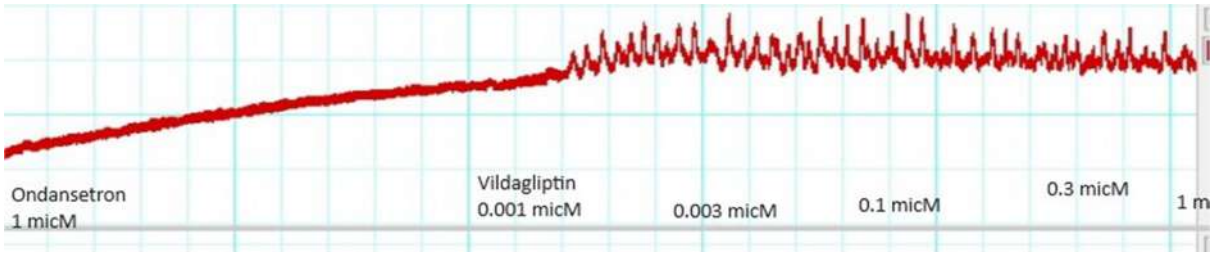

Fig 10: Tracing showing the effect of increasing concentration of vildagliptin in the presence of a fixed concentration of ondansetron.

The cumulative dose-response curve for increasing concentrations of vildagliptin alone was plotted and the response was depicted as a change in the force of contraction on rabbit ileum and rat gastric fundus tissue. There was an inhibitory response as shown by a change in mean concentration from 15.02±2.08 to 13.72 ±2.034 on rabbit ileum and from 7.97 ±1.607 to 7.7 ± 1.5 on rat gastric fundus tissue.

The cumulative dose-response curve of increasing concentrations of vildagliptin was plotted in the presence of a fixed concentration of ondansetron on rabbit ileum and rat gastric fundus tissue . An inhibitory response was observed in intestinal motility from 15.95±3.04 to 14.2±2.9 on rabbit ileum and from 7.6±1.58 to 7.5±1.56 on rat gastric tissue .

An unpaired t-test was used to compare the means of the effects of vildagliptin alone and vildagliptin in the presence of a fixed concentration of ondansetron. A p-value < 0.0001 showed a significant difference between the two groups. When we compared the effect of sitagliptin alone ,sitagliptin on ondansetron, vildagliptin alone and vildagliptin on ondansetron by using one way ANOVA highly significant result was observed between the groups (p-value <0.0001).

DISCUSSION

This study was designed to evaluate the effect of DPP-4 inhibitors (sitagliptin, vildagliptin) on various GI tissues such as rabbit ileum and rat gastric fundus tissue alone and in the presence of ondansetron.

Sitagliptin is a dipeptidyl peptidase 4 inhibitor used for the treatment of diabetes mellitus. This drug causes an increased level of GLP-1 in the body which acts on GLP-1-like receptors to increase insulin secretion and hence control blood glucose level. In this study, an increasing concentration of sitagliptin was administered on rabbit ileum and rat gastric fundus tissue to observe their effects. There was a dose-dependent inhibitory effect exerted by sitagliptin on the intestinal motility of rabbit ileum and rat gastric fundus tissue. Sitagliptin induces dose-dependent relaxation through the activation of PKA and the opening of potassium channels. These results are consistent with the study done by Li et al.[23] Maner also documented the smooth Muscle relaxant effect of sitagliptin on the thoracic aorta of animal models[24].

The effects of increasing the concentration of sitagliptin in the presence of a fixed dose of ondansetron were also explored. There was an inhibitory effect demonstrated at lower concentrations but at higher concentrations there was a stimulatory effect on intestinal as well as gastric contractility. The reason for this effect might be the synergistic effect along with ondansetron at higher doses. This effect might be due to the direct release of serotonin by high doses of sitagliptin in the presence of ondansetron.[12,13]

Maerin also documented the excitatory effect of sitagliptin in his study[25]. This dual effect of sitagliptin needs further exploration regarding its mechanism.

Vildagliptin was administered in increasing concentration and there was mild inhibitory effect on intestinal motility observed at higher doses. This inhibition of smooth muscle contractility is attributed to the opening of potassium channels and SERCA pumps as suggested by Jung et al.[26]

Increasing concentrations of vildagliptin in the presence of ondansetron showed an inhibitory effect at higher concentrations. No significant difference was observed between the effects of increasing concentrations of vildagliptin alone and increasing concentrations of vildagliptin in the presence of ondansetron on rat gastric fundus tissue.

The current investigation affirms that sitagliptin, both alone and at low dose in combination with ondansetron, inhibits the motility of rabbit ileum and rat gastric fundus tissue. Conversely, Vildagliptin does not impact much the intestinal motility of rabbit ileum and rat gastric fundus tissue independently or in conjunction with ondansetron. Both vildagliptin and sitagliptin are DPP-4 inhibitors but their in vitro effects were variable showing inter-drug variation.

Vildagliptin is a relatively safe option for patients with diabetic gastroparesis, as it has minimal impact on intestinal motility. On the other hand, using sitagliptin for treatment requires caution due to its tendency to slow down Gastrointestinal motility as there will be an additive effect due to the in Vivo production of GLP-1 which is known to reduce gastric motility.

A combination of a high dose of sitagliptin and ondansetron has a synergetic stimulatory effect. This combination may cause diarrhoea. Also, this combination may be helpful in the treatment of diabetic enteropathy manifested as gastroparesis and constipation. The mechanism underlying this effect needs further exploration. The combined effect of high doses of sitagliptin and vildagliptin in the presence of a fixed concentration of ondansetron needs further validation.

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