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Unveiling the Differential Impact of Clarithromycin and Metronidazole on the Incretin Effect of Sitagliptin

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ABSTRACT

Background: Sitagliptin is used in the management of type 2 diabetes mellitus. **Metronidazole** is frequently administered in patients prone to severe complications following amoebic infections. **Objective:** The study explores the effects of sitagliptin in conjunction with clarithromycin and metronidazole, the principal medications indicated for amoebic infections. **Method:** Male Wistar rats were administered oral doses of 10 mg/kg body weight of sitagliptin, 10 mg/kg body weight of clarithromycin & 5 mg/kg body weight of metronidazole, individually and in combination over a 14-day period. Weekly blood glucose levels were monitored using a glucometer, while plasma insulin was assessed via heart puncture and analyzed using ELISA. **Results:** The study revealed that while clarithromycin and metronidazole independently exerted no hypoglycemic impact, clarithromycin, when paired with sitagliptin, enhanced its anti-diabetic efficacy, indicating a drug-drug interaction. After evaluation of liver drug-metabolizing enzyme it was found that this interaction was attributed to clarithromycin's inhibitory effect on the CYP3A4 enzyme, responsible for metabolizing sitagliptin, thereby augmenting its anti-diabetic action. Conversely, no such interaction was observed when metronidazole was co-administered with sitagliptin. **Conclusion:** The findings of this study suggest the potential need for dose modification and caution when prescribing a combination of clarithromycin and sitagliptin, whereas the concurrent use of metronidazole and sitagliptin appears to have negligible influence on the therapeutic efficacy of sitagliptin.

Keywords: Sitagliptin, metronidazole, clarithromycin, OGTT (Oral glucose tolerance test), ELISA (Enzyme link Immune sorbent assay) GLP-1(glucagon like peptide-1).

INTRODUCTION

Diabetes mellitus, a persistent metabolic disorder **characterized by** high blood sugar level [1]. This abnormal elevation in blood glucose levels can be attributed to either a deficiency in insulin production or to a diminished response of the body's cells to insulin [2]. Recent global estimates suggest that a staggering 422 million individuals are grappling with diabetes mellitus worldwide, a number that is projected to escalate to around 438 million by the year 2030 [3]. Positioned seventh globally in terms of prevalence, Pakistan's population exhibits a diabetes incidence rate ranging between 7.6 and 11 percent [4].

Incretins, integral metabolic hormones generated by the gastrointestinal tract, modulate insulin secretion through a glucose-dependent process [5]. After ingestion of food the GIT secret the incretins in blood which augment the secretion of insulin from pancreatic beta cells of the islets of Langerhans by a blood glucose-dependent mechanism [6]. Incretins also decreased the

absorption of glucose into the blood by decreasing gastric emptying and inhibits the glucagon secretion from the pancreatic alpha cells [7] .The two main human incretins are GLP-1(glucagon like peptide-1) and glucose-dependent insulin tropic peptide (GIP) [8].In healthy humans, the effects of GIP and GLP-1 account for 25–70% of the total postprandial insulin response (depending on the size of the glucose load), but in type-2-diabetes, the incretin effect is greatly reduced or lost. The loss is a specific and early characteristic of type-2-diabetes development and is largely due to a reduction in the insulin tropic effect of both hormones, although an impairment in the secretion of GLP-1 may contribute[7, 9].The incretin hormones (GLP-1 and GIP) are degraded by an enzyme dipeptidyl peptidase-4 which lead to diminish the effect of incretins hormones [10].

Oral antidiabetic agents such as Dipeptidyl peptides-4 inhibitors are used in the treatment of type-2 diabetes, amongst which sitagliptin is most prescribed [11]. Sitagliptin inhibit the Dipeptidyl peptides-4 which are responsible for the degradation of incretin hormones [12]. Dipeptidyl peptidase-4 inhibition increases the level of incretin. These incretins stimulate secretion of insulin from beta cells [13]. Studies shows that sitagliptin have drug interaction with other drugs [14].

As the number of medications prescribed to patients escalates, the probability of pharmacological interactions correspondingly intensifies [15]. Investigating drug interactions holds a critical significance, particularly with respect to pharmaceuticals characterized by a narrow therapeutic index and those that necessitate prolonged administration. Evidence suggests a considerable susceptibility amongst diabetic individuals to both protozoal and bacterial infections, which subsequently can precipitate conditions such as amoebiasis and ulceration. [16the administration of anti-amoebic pharmaceuticals, specifically metronidazole and clarithromycin, is typically recommended [17]. The integrative therapeutic approach, combining sitagliptin with clarithromycin and metronidazole, for diabetic patients battling both amoebiasis and ulcer, precipitates an intriguing pharmacological interaction [18]. This study was designed to evaluate the effect of clarithromycin and metronidazole on the activity and pharmacokinetic of sitagliptin to evaluate the safety and effectiveness of the combination in animal models.

MATERIALS AND METHODS

2.1. Drugs and chemicals

Sitagliptin, clarithromycin and metronidazole were obtained from Feroz Sons and Zanta Pharma Peshawar Pakistan .Glucose kits and glucometer were purchased from Khyber diagnostics Peshawar, Pakistan.

2.2. Preparation of drugs solutions

2.2.1. Preparation of Clarithromycin & metronidazole suspensions

Required amount of clarithromycin and metronidazole were weighted, triturated separately using mortar and pestle. Required amount of Tween 80 were added as a wetting agent and triturated it well, so that the whole compound was wet, then0.5% of methyl cellulose was added in gravimetric dilution method and triturated uniformly. This suspension was administered to the respective groups of animals through oral gavage.

2.2.2. Preparation of sitagliptin solution

Required amount of sitagliptin was weighed and dissolved it in distilled water through continuous shaking.

2.3. Experimental animals

Male Wistar rats of age 10 to 12 weeks and weigh between 200 to 250 g, were used in the study. Animals are purchased from National Institute of Health Islamabad; Pakistan. The animals were kept in respective cages in the animal house of Khyber Medical University under standard husbandry conditions at an ambient temperature of 25 ± 2ºC and 50 ± 15% relative humidity, with a 12-hr light/dark cycle for at least one week before conducting experiment. Animals were feed with commercial pellet diet and distilled water. The experimental work was performed after approval from the Ethical Board of the Khyber Medical University under application no (Reg.No DIR/KMU/-ED/EC/00435). The study was conducted in accordance with the guidelines provided by the committee for the purpose of control and supervision of experiments and animals.

2.4. Experimental design

Total 35 rats were used. The rats were randomly divided into 7 groups (each group contain 5 rats). The drugs were administered to the respective groups by oral gavage according to the following methods.

Group 1: Normal control (only food was given). Group 2: Sitagliptin (10 mg/kg).

Group 3: Clarithromycin (10 mg /kg). Group 4: Metronidazole (5 mg /kg).

Group 5: Sitagliptin (10 mg/kg) and clarithromycin (10 mg/ kg).

Group 6: Sitagliptin (10 mg /kg) and metronidazole (5 mg /kg).

Group 7: Sitagliptin (10 mg /kg) and metronidazole (5 mg /kg), clarithromycin (10 mg/ kg).

2.5. Biochemical Parameter

2.5.1. Blood glucose level

Blood glucose level was measured with the help of glucometer (Abbott Freestyle) at weekly intervals (1, 7and 14) days after daily administration of drugs by oral gavage.

2.5.2. Oral glucose tolerance test

After 14 days of drugs administration all the rats were kept on fasting for 24hr. than 400 mg glucose was given to each group orally and blood glucose level was measure with the intervals of 30, 60 and 120 minutes through glucometer.

2.5.3. Blood collection

After OGTT the whole blood was collected by cardiac puncture while giving little anaesthesia by using diethyl ether to the rats. The blood was collected in heparinised tube. The plasma was separated by centrifugation with the help of research centrifuge at 4000rpm for 10 to 20 minutes. The plasma was stored under -20C. This plasma was used for further determination of insulin test both in control and experiential rats by using the insulin ELISA kits.

2.5.4. Plasma insulin

The process of quantifying plasma insulin involved the collection of blood specimens in heparin tubes. The plasma component was subsequently isolated and preserved at -20 degrees Celsius pending insulin assay. The plasma insulin assay was carried out using rat specific insulin ELISA kit according to manufacturer's instruction. After preparing the reagents, each well was added 25 microliters of sample and standard followed by the addition of 100 microliter of insulin enzyme conjugate and incubated for 1h at ambient temperature, concurrently undergoing a gentle shaking motion at a frequency between 700 to 900 revolutions per minute on a microplate shaker. Following incubation, the wells were emptied and rinsed thrice, each time with 300 microliters of a washing buffer solution. Subsequently, 100 microliters of a 3,3,5,5-tetramethylbenzidine (TMB) substrate were introduced into each well, then incubated for 15 min at room temperature in the dark and followed by the addition of 50 microliter stop solution. This resulted in a gentle agitation of the plate to facilitate mixing. Finally, after a further 15-minute interval post the addition of the stop solution, we conducted a measurement of absorbance. Using a microplate reader (Biotech, Winooski, VT), the absorbance was read on the ELISA plate at a wavelength of 450 nm, yielding the insulin quantification necessary for our analysis.

RESULTS

3.1. Effect of sitagliptin with clarithromycin and metronidazole on blood glucose level

As described in Figure.1, the combined use of sitagliptin with clarithromycin & metronidazole caused a significant decreased in blood glucose level, $p < 0.05$ as compared to control group, which is 117.4 ± 3.21mg/dl and 99 ± 3.21mg/dl for control and (sitagliptin, clarithromycin and metronidazole) groups respectively.

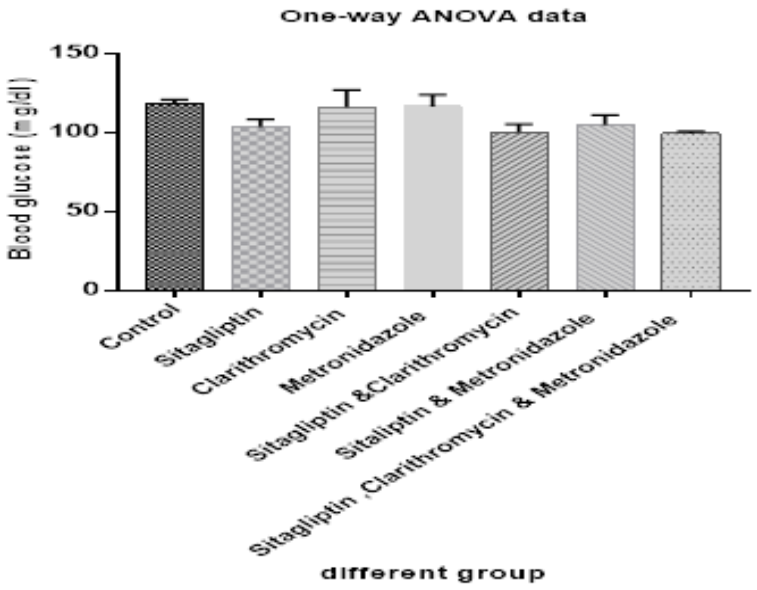

Figure 1. Effect of sitagliptin with clarithromycin & metronidazole on blood glucose level.

3.2. Effect of sitagliptin with clarithromycin and metronidazole on plasma insulin level

As shown in Figure.2, sitagliptin, clarithromycin and metronidazole caused significant increase in plasma insulin level, $p < 0.05$ as compared to control group which is 10.37 ± 1.54 µlU/ml and 17.77 ± 1.04 µlU/ml for control and (sitagliptin, clarithromycin and metronidazole) respectively.

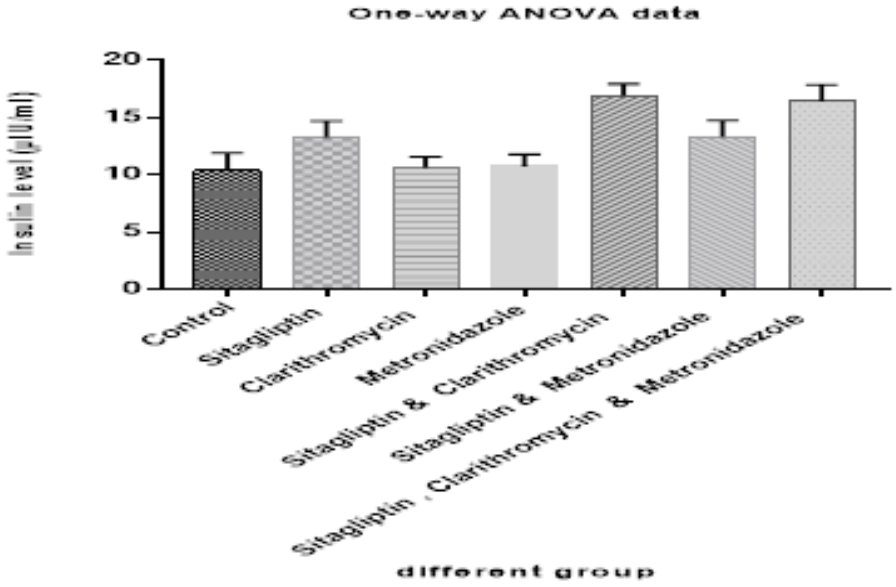

Figure 2. Effect of sitagliptin with clarithromycin & metronidazole on plasma insulin level

3.3. Oral glucose tolerance test

After 14 days of treatment all animals were keep for 24 h on fasting and then administer 0.4 g/kg of oral glucose to both normal and treated groups. Blood glucose levels of all animal were measured with the help of Glucometer at 0, 30, 60 and 120 minutes. The results of OGTT (Oral glucose tolerance test) is explained in Figure.3..

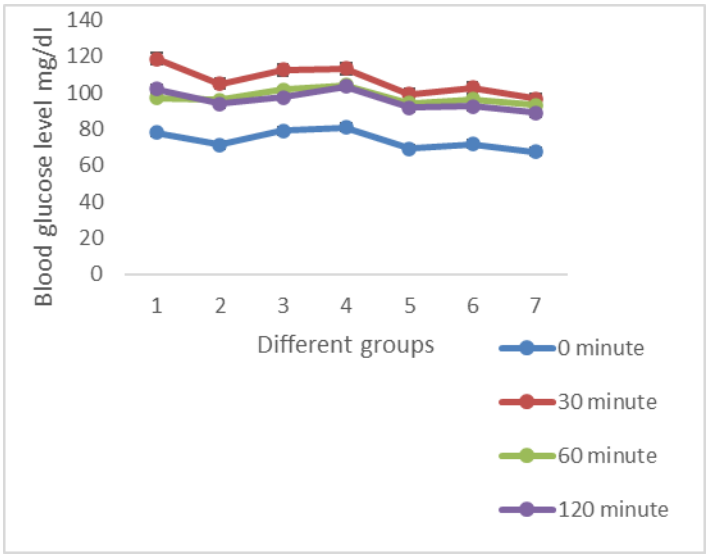

Figure 3. Show the oral glucose tolerance test of control and treated animals at 0, 30, 60 and 120 minutes.

Control-1, Sitagliptin-2, Clarithromycin-3, Metronidazole-4, Sitagliptin & clarithromycin-5, Sitagliptin & metronidazole-6, Sitagliptin, clarithromycin and metronidazole-7.

DISCUSSION

In the realm of clinical practice, drug interactions are a prevalent phenomenon, with their underlying mechanisms typically deciphered through the utilization of animal models. Our research delved into the influence of clarithromycin and metronidazole, two antibiotics, on the pharmacodynamic profile of sitagliptin at doses typically prescribed in a therapeutic setting, utilizing healthy male Wistar rats as our experimental model. Sitagliptin, an antidiabetic agent, elicits hypoglycemic effects by promoting insulin release from pancreatic β cells, a process facilitated by the inhibition of the DPP-4 enzyme. Notably, patients with diabetes often exhibit heightened susceptibility to bacterial and protozoal infections, leading to conditions such as amoebiasis and peptic ulcer disease [19]. In these cases, metronidazole and clarithromycin are most commonly prescribed [20]. The concomitant administration of clarithromycin and metronidazole appears to potentiate the antidiabetic efficacy of sitagliptin, thereby signifying a notable drug-drug interaction between these medicinal agents. This study, conducted over a fortnight, demonstrated notable disparities in the blood glucose and plasma insulin levels between the control group and the various treated cohorts. Sitagliptin, in particular, elicited a significant reduction in blood glucose levels by 16.32% relative to the control group. This decline in blood glucose can be attributed to a corresponding 22% surge in plasma insulin levels in the sitagliptin-treated group. It is noteworthy that clarithromycin and metronidazole, in isolation, did not significantly impact blood glucose and plasma insulin levels. Yet, an intriguing trend emerged with the combined therapy of sitagliptin and clarithromycin. This combination markedly impacted both blood glucose and plasma insulin levels when contrasted against the control group and the group treated with sitagliptin alone. This combination therapy led to a decrease in blood glucose levels by up to 17.16%, coupled with a dramatic increase in plasma insulin levels by up to 38.63%. In contrast, no significant disparities were observed when comparing the effects of sitagliptin alone versus sitagliptin in conjunction with metronidazole. Further, the concurrent use of the three drugs — sitagliptin, clarithromycin, and metronidazole — did not significantly deviate from the results observed in the sitagliptin plus clarithromycin group. This suggests that the drug-drug interaction predominantly exists between sitagliptin and clarithromycin. (Figure1.2).

Hence, the current study elucidates that co-administration of sitagliptin with clarithromycin and metronidazole can instigate drug interactions. It was observed that the therapeutic intervention involving clarithromycin significantly modulates the pharmacodynamic profile of sitagliptin, potentiating its antidiabetic effects. This implies that clarithromycin exerts a delaying impact on the metabolism of sitagliptin, chiefly by inhibiting the enzymatic components responsible for its metabolic breakdown. Numerous scholarly articles have underscored that sitagliptin is primarily metabolised by the CYP3A4 and CYP2C8 isozymes [21]. Concurrently, it has been well documented in previous literature that clarithromycin possesses the ability to inhibit these specific enzymes, namely CYP3A4 and CYP2C8 [22]. Thus, the empirical evidence garnered from this study distinctly demonstrates the occurrence of drug interactions when sitagliptin and clarithromycin are concomitantly administered.

Given these findings, it is imperative to implement rigorous patient monitoring strategies may be adopted to avert potential dysglycemia when this therapeutic combination is prescribed. This not only ensures optimal therapeutic outcomes but also significantly mitigates the risk of adverse metabolic implications.

Conclusion

In conclusion, this study confirms the existence of a pharmacological interaction between clarithromycin and sitagliptin. It is noteworthy that clarithromycin potentially potentiates the hypoglycemic efficacy of sitagliptin, thereby necessitating a careful reconsideration of dosage when these two drugs are co-administered. Further, it is critical to proceed with caution while prescribing them concurrently to mitigate potential complications. Interestingly, our findings also establish that sitagliptin does not exhibit any discernible pharmacological interaction with metronidazole. This study serves as an important step in understanding the nuanced dynamics of drug interactions, paving the way for safer and more effective clinical prescriptions.

Acknowledgement

N/A

CONFLICT OF INTEREST

For the current study, the contributing authors herein unequivocally affirm the absence of any potential conflicts of interest.

AUTHOR CONTRIBUTION

Each author has made an equal and substantial intellectual contribution to this work, encompassing the conceptualization, data acquisition, and analysis. Furthermore, they have been intimately involved in the drafting and critical revision of the manuscript, thereby satisfying the requisite criteria for authorship.

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