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Original Article

PHARMACOKINETIC DRUG INTERACTIONS OF GLICLAZIDE AND ITOPRIDE IN NORMAL AND DIABETIC RATS

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ABSTRACT

Objective: The present study was aimed to investigate the safety, reliability of Gliclazide and possible drug interaction with Itopride when they were administered as combination treatment.

Methods: Studies were conducted in normal and streptozotocin induced diabetic rats with oral administration of selected doses of gliclazide, itopride and their combination. Blood samples were collected from rats by retro orbital/marginal ear vein puncture at regular intervals of time. All the blood samples were analyzed for pharmacokinetic parameters by HPLC method.

Results: There was no significant difference in pharmacokinetic parameters of both Gliclazide alone and combination with itopride in healthy and diabetic rats on day 1 and day 8.

Conclusion: Based on the results it can be concluded that the concurrent administration of these two drugs have potential benefit without any drug interactions in the effective management of diabetes and gastroparesis.

Keywords: Drug interactions, Gliclazide, Itopride, Pharmacokinetics, Gastroparesis, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a metabolic disease in which there are high blood sugar levels over a prolonged period. Diabetes mellitus is due to the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [1].

Normally, the muscles of the stomach, which are controlled by the vagus nerve, contract to break up food and move it through the gastrointestinal (GI) tract. Diabetes is the most common known cause of gastroparesis. People with diabetes have high levels of blood glucose, also called blood sugar. Over time, high blood glucose levels can damage the vagus nerve.

Gastroparesis is a syndrome characterized by delayed gastric emptying [2], in the absence of mechanical obstruction of the stomach. The cardinal symptoms include postprandial fullness (early satiety), nausea, vomiting and bloating [3]. In one tertiary referral series, diabetes accounted for almost one third of cases of Gastroparesis [4]. Diabetes patients with gastroparesis develop retinopathy, neuropathy, nephropathy, nutritional compromise, impaired glucose control, and a poor quality of life [5].

Symptoms attributable to gastroparesis are reported by 5 to 12% of patients with diabetes [6, 7, 8, and 9]. Gliclazide is an oral hypoglycemic (anti-diabetic drug) and is classified as a sulfonylurea. Its classification has been ambiguous, as literature uses it as both a first-generation [10], and second-generation [11] sulfonylurea. Gliclazide was shown to protect human pancreatic beta-cells from hyperglycemia-induced apoptosis [12]. It was also shown to have an antiatherogenic effect (preventing accumulation of fat in arteries) in type 2 diabetes [13]. Itopride is a prokinetic benzamide derivative unlike metoclopramide or domperidone. These drugs inhibit dopamine and have a gastro kinetic effect [14]. Itopride is indicated for the treatment of functional dyspepsia and other gastrointestinal conditions [15]. After oral administration Itopride undergoes rapid and extensive absorption with levels of itopride peaking in the blood plasma after only 35 min. Itopride is primarily eliminated via the kidneys having an elimination half-life of approximately 6 h [16].

To treat the coexisting disease, multidrug therapy is inevitable and there is every possibility for a drug interaction to occur when drugs are concomitantly used [17].

MATERIALS AND METHODS

Materials

Drugs and chemicals

Gliclazide and Itopride were procured from Matrix laboratories as a gift sample. Streptozotocin (STZ) was purchased from Sigma Chemical Co. The glucose estimation (GOD-POD) kit (Excel diagnostic, Hyderabad) was procured from the drug store. All HPLC grade solvents (methanol and water) were procured from Finar chemicals Ltd., Ahmadabad. All chemicals used were analytical grade.

Methods

Animal study

Twenty four healthy Wistar rats were (Weighing 200-220 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25 °C, Relative Humidity 45% and 12 h alternate lights and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee (IAEC NO: P16/VCP/IAEC/2012/3/VVR/AE2).

Induction of experimental diabetes

Male Wistar rats (200-250 gms) were fasted for 16 h before the induction of diabetes with Streptozotocin (STZ), Animals (n=6) were injected intra peritoneal with 0.22-0.25 ml of freshly prepared solution of STZ (60 mg/ml in 0.01 m citrate buffer, pH 4.5) at a final dose of 60 mg/kg body wt. The diabetic state was assessed in STZ-treated rats by measuring the non-fasting serum glucose concentration after 48 h. Only rats with serum glucose levels greater than 300 mg/dl were selected and used in this experiment.

Study design [18]

The rats were grouped as follows

Group I: Gliclazide (30 mg/kg, oral) alone in single dose/day in diabetic rats up to 8 d.

Group II: Itopride (20 mg/kg, oral) alone in single dose/day in diabetic rats up to 8 d.

Group III: Itopride (20 mg/kg, oral) alone in single dose/day in normal healthy rats up to 8 d

Group IV: Gliclazide (30 mg/kg, oral) and Itopride concomitant administration in diabetic rats as Single dose/day up to 8 d.

Collection of blood samples

Blood samples were collected after administration of Gliclazide 30 mg/kg, Itopride 20 mg/kg as per animal body weight on day 1 and day 8 blood samples of 1 ml were drawn from each anesthetized (isoflurane) rat at pre-determined time intervals was collected from the retro-orbital plexus using a capillary tube into pre-labeled eppendorf tubes containing 10% of K_2EDTA anticoagulant (20 µl). The time intervals for the sample collection were 0 (Pre dose), 0.5, 1, 2, 4, 6, 8 and 24 h (post dose), Equal amount of saline by oral route was administered to replace blood volume at every blood withdrawal time.

Plasma was obtained by centrifuging blood samples by using refrigerated (REMI ULTRA) at 3000 rpm for 5 min. The obtained plasma samples were transferred into pre-labeled micro centrifuge tubes and stored at −2 0 °C until analysis of pharmacokinetic parameters. As described above, all the procedures were followed on day 8 also. Pharmacokinetic parameters were calculated by non-compartmental analysis by using Win Nonlin5.1 software [19, 20].

Method of analysis

Preparation of plasma samples for HPLC analysis

Rat plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000–6000 rpm for 10 min), acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µ1 of mobile phase were injected for HPLC analysis.

For HPLC analysis C18 column with 5µm particle size, mobile Phase consisting of 0.05M Potassium Dihydrogen phosphate and 0.5% Tri Ethyl Amine (TEA) buffer having pH 2.7, and Methanol in the ratio of 60:40 v/v. The flow rate was 0.8 ml/min and the effluents were monitored at 238 nm. Internal standard Phenformin was used. The retention time of an internal standard (phenformin), gliclazide and itopride in plasma were 9.8, 7.2 and 3.3 min, respectively [21].

Standard calibration curve of Gliclazideand Itopride in rat plasma

Different concentrations (0.05, 0.1, 0.5, 1, 5, 10, 20, 40 µg/ml) of gliclazide, itopride in plasma were prepared for calibration curve. The samples were treated as above for protein precipitation method and peak areas of gliclazide and Itopride were noted down. The peak area ratios obtained at different concentrations of the gliclazide and Itopride were plotted using UV–Vis detector at 238 nm.

Pharmacokinetic analysis

Plasma concentration versus time data was analyzed using standard non-compartment analysis. $AUC_{0\text{-}t}$ refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0\text{-}\alpha}$ refers to the AUC from time at zero hours to infinity. The $AUC_{0\text{-}\alpha}$ was calculated using the formula $AUC_{0\text{-}t}+[C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant. Various pharmacokinetic parameters like area under the curve [AUC], elimination half life [t½]. Apparent volume of distribution (V/f) total clearance (Cl/f) and mean residence time for each subject using a non-compartmental analysis by using Win Nonlin 5.1 software.

Statistical analysis

Statistical comparisons for the pharmacokinetic study among, gliclazide, itopride, itopride alone and in combination groups and plasma concentration–response study among concentrations and time were carried out with student's paired T-Test a value of $P<0.05$ was considered to be statistically significant. Data were reported as mean±SEM linear regressions were used to determine the relationship between total plasma concentrations and pharmacokinetic parameters. The mean concentration versus time profile of gliclazide and itopride in rat plasma is shown in fig. 1, 2, 3 and 4.

RESULTS AND DISCUSSION

In the present study, Gliclazide is completely absorbed after oral administration with peak plasma concentration of 11.54±2.14µg/ml after 2 h of dosing on day 1. In combination with Gliclazide and Itopride on day 1, the peak plasma concentration of Gliclazide 18.33±0.12µg/ml occurred at 2hr after dosing. There was no significant increase in peak plasma concentration levels. Similarly Itopride is completely absorbed after oral administration with peak plasma concentration 3.92±0.03µg/ml occurred at 2hr after dosing on day 1 in combination with Gliclazide and Itopride on day 1.

Table 1: Pharmacokinetic parameters of Gliclazide alone and in Combination with Itopride on day 1

parameters	Gliclazide alone	Gliclazide combination with Itopride	Level of significance($p<0.05$)
C_{max} (µg/ml)	11.54±2.14	18.33±0.12	NS
t_{max} (h)	2±0	2±0	NS
$AUC_{o\text{-}t}$ (µg/ml/h)	137.22±0.74	176.37±0.52	NS
$AUC_{o\text{-}inf}$ (µg/ml/h)	158.82±2.50	158.15±1.85	NS
t½ (h)	11.56±0.28	10.64±0.09	NS
CL/f (ml/h/kg)	1.18±0.015	2.23±0.01	NS
V/F (ml/kg)	25.28±0.26	37.56±0.25	NS

NS: Not Significant

Table 2: Pharmacokinetic parameters of Gliclazide alone and in Combination with Itopride on day 8

Parameters	Gliclazide alone	Gliclazide combination with Itopride	Level of significance ($p<0.05$)
C_{max} (µg/ml)	25.83±1.11	25.41±1.21	NS
t_{max} (h)	2±1.26	2±1.14	NS
$AUC_{o\text{-}t}$ (µg/ml/h)	156.371.24±	158.8±1.21	NS
$AUC_{o\text{-}inf}$ (µg/ml/h)	178.15±1.45	196.12±1.31	NS
t½ (h)	8.78±1.54	9.08±1.41	NS
CL/f (ml/h/kg)	1.961±0.01	1.92±1.51	NS
V/F (ml/kg)	26.67±0.52	27.89±1.25	NS

NS: Not Significant

Table 3: Pharmacokinetic parameters of Itopride in diabetic versus healthy male Wistar rats on day 1

Parameters	Itopride in diabetic rats	Itopride in healthy rats	Level of significance (p<0.05)
C_{max}(µg/ml)	3.92±0.07	2.72±0.03	NS
t_{max}(h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	42.49±11.1	21.9±0.118	NS
AUC_{o-inf} (µg/ml/h)	59.53±41.7	27.49±0.808	NS
t½ (h)	12.22±15.1	12.03±1.11	NS
CL/f(ml/h/kg)	15.475±12.2	26.71±0.18	NS
V/F(ml/kg)	459.16±2.1	497.65±3.09	NS

NS: Not Significant

Table 4: Pharmacokinetic parameters of Itopride in diabetic versus healthy male Wistar rats on day 8

Parameters	Itopride in diabetic rats	Itopride in non diabetic rats	Level of significance (p<0.05)
C_{max}(µg/ml)	4.80±0.04	3.65±0.06	NS
t_{max}(h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	44.11±0.225	38.22±0.09	NS
AUC_{o-inf} (µg/ml/h)	61.57±0.43	67.37±0.336	NS
t½ (h)	12.49±0.186	18.92±0.009	NS
CL/f(ml/h/kg)	13.01±0.08	11.88±0.042	NS
V/F(ml/kg)	255.8±2.192	345.6±0.639	NS

NS: Not Significant

Table 5: Pharmacokinetic parameters of Itopride alone and in Combination with Gliclazide in diabetic rats on day 1

Parameters	Itopride alone	Itopride with Gliclazide	Level of significance(p<0.05)
C_{max}(µg/ml)	3.92±0.03	4.06±0.03	NS
t_{max}(h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	33.5±0.20	41.63±0.20	NS
AUC_{o-inf} (µg/ml/h)	52.6±0.52	52.16±0.525	NS
t½ (h)	15.23±0.27	12.57±0.27	NS
CL/f(ml/h/kg)	15.43±0.26	19.03±0.26	NS
V/F(ml/kg)	358.65±4.31	337.66±4.31	NS

NS: Not Significant

Table 6: Pharmacokinetic parameters of Itopride alone and in Combination with Gliclazide in Diabetic rats on day 8

parameters	Itopride alone	Itopride with Gliclazide	Level of significance(p<0.05)
C_{max}(µg/ml)	4.80±0.04	5.07±0.04	NS
t_{max}(h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	44±0.225	51.6±0.25	NS
AUC_{o-inf} (µg/ml/h)	61.56±0.43	69.76±0.88	NS
t½ (h)	12.49±0.18	12.89±0.29	NS
CL/f(ml/h/kg)	13.01±0.08	13.48±0.03	NS
V/F(ml/kg)	335.89±2.16	371.1±2.26	NS

NS: Not Significant

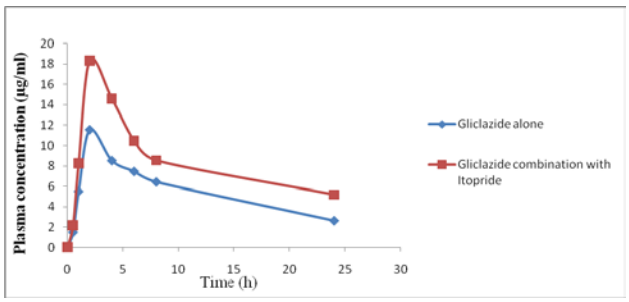

Fig. 1: Mean Plasma concentrations (µg/ml) of Gliclazide alone and combination with Itopride on day 1 in diabetic male Wistar rats (n=6)

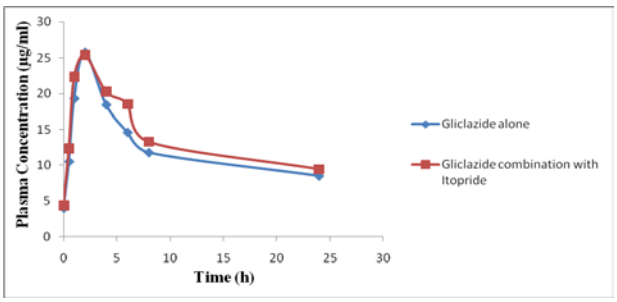

Fig. 2: Mean Plasma concentrations (µg/ml) of Gliclazide alone and combination with Itopride on day 8 in Diabetic male Wistar rats (n=6)

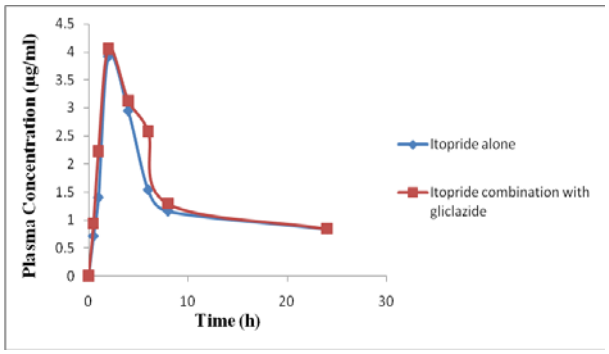

Fig. 3: Mean plasma concentrations (µg/ml) of Itopride alone and combination with Gliclazide on day 1 in diabetic male Wister rats (n=6)

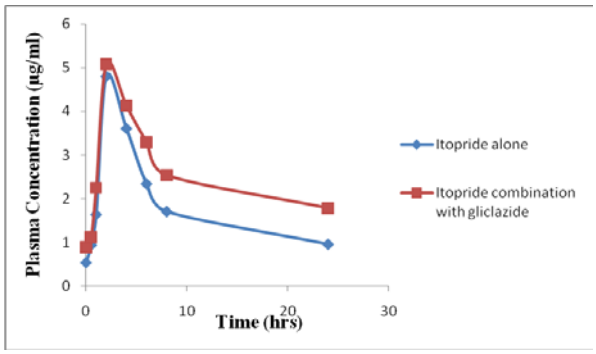

Fig. 4: Mean plasma concentrations (µg/ml) of Itopride alone and combination with Gliclazide on day 8 in diabetic male Wister rats (n=6)

The peak plasma concentration, of Itopride 4.06±0.03µg/ml occurred at 2hr after dosing. There was no significant increase in the peak plasma concentration levels similarly on day 8 of Gliclazide alone and with combination of Gliclazide with Itopride on day 8. Peak plasma concentration are 25.83µg/ml and 25.41µg/ml respectively similarly Itopride on day 8 and combination with Gliclazide concentrations are 4.80± 0.04 µg/ml and 5.07 ± 0.04µg/ml respectively. There was no significant difference in peak plasma concentration on day 8 ($P>0.05$). A slight differences was observed between diabetic and healthy Itopride treated rats on day 1 and day 8 respectively ($P<0.05$) on oral administration of Itopride alone and with the combination of Gliclazide. With Itopride on day 1 showed a 2% increase in the AUC $_{0-24}$ of Gliclazide compared to combinational treatment, similarly. Itopride on day 1 and with combination Gliclazide with Itopride on day 1 administration resulted in an increase in the AUC $_{0-24}$ of Itopride compared with combinational treatment. Similarly on day 8 of Gliclazide and Itopride in combination treatment were 1.65% and 2.8% increase in the AUC$_{0-24}$ respectively. The mean AUC$_{0-24}$ of Itopride in Diabetic rats was 33.49± 0.20 µg/ml/h and 44.11 ±0.22µg/ml/h which was reduced to 21.9±0.11µg/ml/h and 38.22 ±0.09µg/ml/h Itopride in healthy rats on day 1 and day 8 treatment ($P<0.05$) respectively. There was the slight decrease in the clearance (CL/F) rate of Gliclazide in combination compared with Gliclazide alone by 2.52% and 0.92% on day 1 and day 8 respectively. Similarly there was the slight decrease in the clearance (CL/F) of Itopride in combination compared with Itopride alone by 4.92% on day 1. On day 8 a slight increase in the clearance (CL/F) of Itopride in combination compared with Itopride alone by 4.6%.

The half life was similar with alone and combination treatment on day 1 and day 8. All these changes were not statistically significant ($P>0.05$). The mean clearance (C1/F) was 15.475±0.401 (ml/h/kg) and 26.71±0.18 which was reduced to 13.01±0.08 (m1/h/kg) and 11.58±0.042 (m1/h/kg) upon treatment of Itopride in Diabetic rats and Healthy rats on day 1 and day 8 respectively.

Volume of distribution was increased 3.8% and 1.72% in Gliclazide alone compared with Gliclazide and Itopride on day 1 and day 8 respectively. similarly Itopride in diabetic rats versus in healthy rats resulted 359.16 and 497.65 on day 1 respectively Itopride alone on day 1 and combination with Gliclazide on day 1 administration resulted in 1.5% increases of volume distribution (m1/kg) in alone Itopride group treated rats similarly on day 8 administration resulted in 5.5 increases of volume of distribution in alone Itopride treated rats. From the above results there is no significant difference of C_{max}, t_{max}, AUC $_{(0-t)}$ and AUC $_{(0-inf)}$, $t_{½}$, Cl/f and V/f on day 1 and day 8 of Gliclazide and Itopride in both diabetic and healthy rats. Potentially the combination use of Gliclazide and Itopride is safe.

CONCLUSION

Considering the prevalence of diabetic gastroparesis as well as the prescribing pattern of drugs in patients suffering from such complications, it is apparent that antidiabetic and gastroparesis drugs seem to be potential candidates for concomitant administration. Gliclazide and Itopride are prescribed frequently together to the diabetic-gastroparesis patients. The present work was aimed to confirm the safety of concomitant administration of Gliclazide and Itopride in rats without any drug-drug interactions. There was no significant change in the serum levels and pharmacokinetics parameters of C_{max}, t_{max}, AUC $_{(0-t)}$ and AUC $_{(0-inf)}$, **$t_{½}$**, Cl/f and V/f on day 1 and day 8 of Gliclazide and Itopride in both diabetic and healthy rats. It may be concluded that during concomitant administration of Gliclazide and Itopride at therapeutic doses, drug–drug interaction does not occur. Therefore, the therapeutic dose and the frequency of administration of Gliclazide need not be adjusted in Gastroparesis patients with Diabetes.

CONFLICT OF INTERESTS

Declare None

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