Short Communication

JYP

Pharmacokinetic Interaction of Gliclazide with Ornidazole in Healthy Albino Wistar Rats

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ABSTRACT

Background: Gliclazide is a widely used drug for the treatment of type 2 diabetes. Retrospective studies shows that diabetics are at increased risk of suffering severe complications after amoebic infection. Ornidazole is a widely used anti-amoebic drug. The objective of this study was to examine the effect of oral administration of ornidazole on blood glucose levels, investigate its effect on the activity and pharmacokinetics of gliclazide in normal rats and to evaluate the safety and effectiveness of the combination. **Methods:** Studies in normal rats were conducted with oral doses of 2 mg/kg body weight of gliclazide and 25 mg/kg body weight of ornidazole andin combination. Blood samples were collected at regular time intervals from rat's orbital sinuses. All the blood samples were analysed for blood glucose by glucose oxidase/peroxidase (GOD/POD) method and HPLC was used to understand the pharmacokinetics of gliclazide alone and in combination with ornidazole. **Results:** The results of the present study is indicating that ornidazole does not possess glucose lowering activity therefore it may be inferred that drug-drug interaction of ornidazole with gliclazide is pharmacokinetic type and the possible interaction may be due to CYP2C9 inhibition. Gliclazide produced glucose lowering activity in normal rats with peak activity at 2 h. In combination, ornidazole increases the effect of gliclazide in rats. **Conclusion:** Thus, it can be concluded that the combination of ornidazole and gliclazide may need slight dose adjustment and care should be taken when the combination is prescribed for their clinical benefit in diabetic patients. However, further studies are warranted.

Key words: Albino wistar rats, Cytochrome P 2C9, Gliclazide, HPLC (High Performance Liquid Chromatography) Ornidazole, Pharmacokinetic interactions.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolisms leading to complications involving many organs. There are estimated 143 million people worldwide sufferings from diabetes[1] and the number may probably double by the year 2030.[2] Diabetes may be due to decrease in the synthesis of insulin (type-1) or decrease in the secretion of insulin (type-2) from the β-cells of islets of langerhans of the pancreas. Oral hypoglycemic agents are used in the treatment of type-2 diabetes, amongst which gliclazide, a second generation sulfonylurea derivative is preferred in the therapy because of its selective inhibitory activity towards pancreatic K^+ATP channels, anti-oxidant property, low

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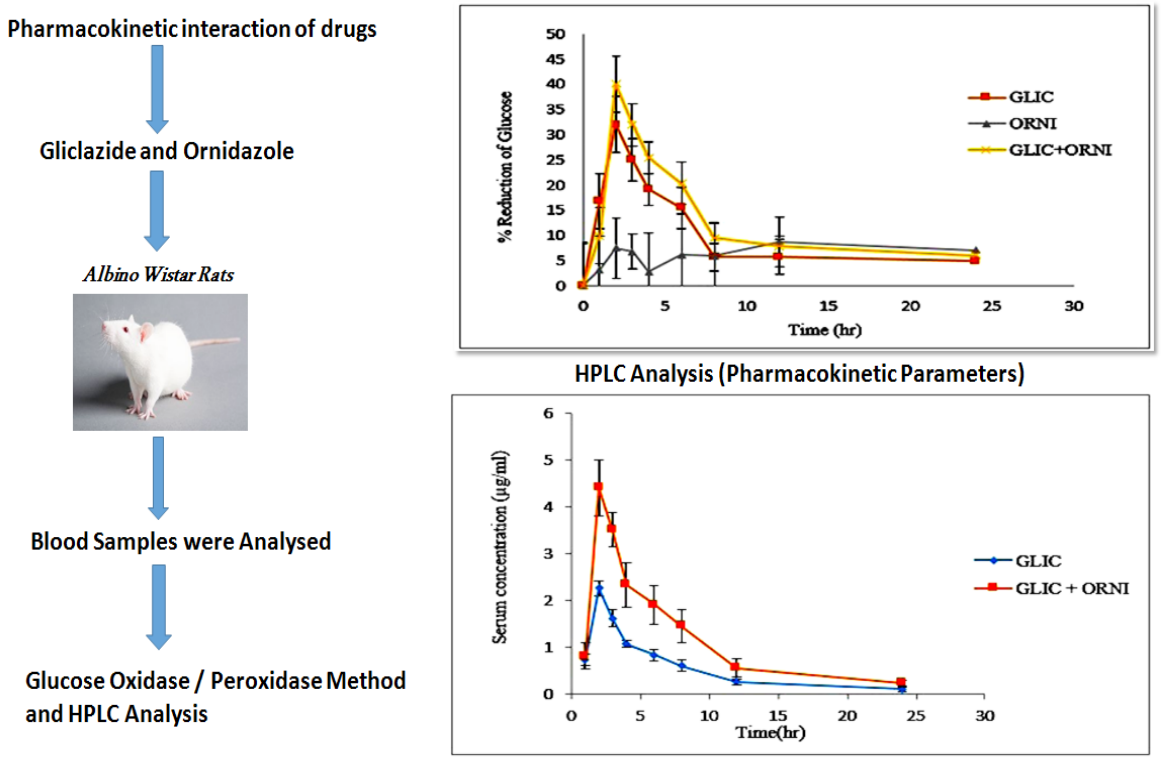

Graphical Abstract

incidence of producing severe hypoglycaemia and other haemo-biological effects.[3] Gliclazide induces the release of insulin by triggering calcium entry into the pancreatic β cells by K^+ ATP channels blockade. Earlier studies indicate interaction of gliclazide and many other anti-diabetic drugs with several other classes of drugs.[4-6]

As the number of drugs in patient's therapeutic regimen increases, greater is the risk of occurrence of drug interaction.[7] Drug interaction studies assume much importance, especially for drugs that have a narrow margin of safety and where the drugs are used for a prolonged period of time. There are reports that several patients suffering from diabetes are prone to protozoal infections like amoebiasis that produced an amoeboma which is most rare in cases of colonic amoebiasis. The clinical picture was that of an occluding gut tumor, which can be treated with drugs.[8] In such case anti-amoebic agents like metronidazole, ornidazole, tinidazole etc., are used.

The concomitant administration of gliclazide with ornidazole in diabetic patients infected with amoebiasis may result in drug-drug interaction. The study is planned to investigate the effect of ornidazole on the activity and pharmacokinetics of gliclazide in order to evaluate the safety and effectiveness of the combination in animal models.

MATERIALS AND METHODS

Drugs and chemicals

Gliclazide (99.8%)was purchased from Dr Reddy's laboratories (Hyderabad, India) whereas ornidazole (99.62%) is obtained from Sri SJS Pharma (Hanamkonda, India). Glucose test kits of Excel Diagnostics are purchased from local suppliers. HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals used were analytical grade.

Animals

Albino rats of either sex, 3-4 months of age, weighing between 200 to 250 g, were used in the study. Animals are procured from Mahaveer Enterprises (Hyderabad, India) and were housed under standard husbandry conditions at an ambient temperature of 25 ± 2°C and 50 ± 15% relative humidity, with a 12-h light/12-h dark cycle for at least one week before conducting experiment. Animals were fed with commercial pellet diet and water ad libitum. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee (Reg. No:1047/ac/07/CPCSEA) in Vaagdevi College Laboratories, (Hanamkonda, India). The study was conducted in accordance with the guidelines provided by

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Pharmacokinetic interaction study in normal albino wistar rats

Gliclazide(2 mg/kg b.wt)[3] and ornidazole (25 mg/kg b.wt)[9] suspensions were prepared using 2% w/v gum acacia as suspending agent. Both the drugs were administered to the respective groups by oral gavage. All the animals were fasted for 18 h prior to the experiment, and water and food are withdrawn during the experiment.

Albino wistar rats of either sex are randomly distributed into 4 groups of 5 animals each.

Group I: Received only vehicle (2% gum acacia)

Group II: Received only Gliclazide (2 mg/kg)

Group III: Received only Ornidazole (25 mg/kg)

Group IV: Received 8 days pre-treatment of ornidazole (25 mg/kg) and on 9th day administration of ornidazole (25 mg/kg) is followed by Gliclazide (2 mg/kg) after 30 minutes.

In this study, after anaesthetising the animals with diethyl ether, the blood samples were collected at 1, 2, 3, 4, 6, 8, 12 and 24 h after treatment from orbital sinuses by retro puncture using heparinised capillaries into centrifugation tubes containing 0.1 mL 0.2% sodium citrate. Plasma was separated by centrifugation and stored under -20^{o}C. These samples are used for both glucose estimation and HPLC analysis. Estimation of glucose levels was done using glucose oxidase/peroxidasekit.

Bioanalytical method

Plasma gliclazide concentrations were determined using Waters 2487 High Performance Liquid Chromatography (HPLC) unit equipped with solvent delivery module LC-20AD, SPD-20A UV detector, Kromasil 100–5C18 column (100 mm × 4.6 mm, 5 µm) and is operated at 230 nm. An isocratic mobile phase consisting of a mixture of acetonitrile, methanol and water (40:35:25, v/v/v) was used to separate the analyte from the endogenous components and delivered at a flow rate of 1.00 mL/min.

All the samples were vortexed to mix for 10 s prior to spiking. A 100 µL aliquot of serum sample was mixed with 50 µL of the internal standard working solution (50 µg/mL of ibuprofen). To this, 1 mL of methanol was added. After vortex–mixing for 60 s and centrifugation at 4000 rpm for 15 min, the supernatant was transferred to a 5 mL glass test tube and evaporated at 45°C under a gentle stream of nitrogen. The dried extract was reconstituted with 100 µL of the methanol and a 50 µL aliquot of it was injected into the HPLC system. Gliclazide and the IS were eluted at 5.7 ± 1 and 8.6 ± 1 min, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters of gliclazide were calculated using and the parameters includes half-life ($t_{1/2}$), clearance (C_l), volume of distribution (V_d), C_{max}, T_{max}, and total area under curve (AUC_{total}).

Data and statistical analysis

Data were expressed as mean ± standard deviation (SD). The significance was determined by applying unpaired t test. ***P<0.0001 and **P<0.0022 were considered significant.

RESULTS

In the present study it was revealed that ornidazole doesnot possess glucose lowering activity. Also, no significant alterations in the plasma blood glucose levels was observed when gliclazide is administered alone and in combination with ornidazole (Table 1) (Figure 1). Figure 1 depicts the % reduction of blood glucose - time profile of gliclazide (2

Table 1: Percentage reduction of blood glucose levels

Time interval (hr)	Ornidazole	Gliclazide	Combination (9th day)
1	3.217±2.17	16.817±8.3	9.950±8.5
2	7.517±6.53	31.962±5.4	40.032±5.5
3	6.759±5.97	24.951±5.5	31.929±5.6
4	2.711±3.45	19.099±4.2	25.484±4.2
6	6.089±7.78	15.427±3.1	20.342±3.1
8	5.877±8.08	5.651±4.0	9.466±4.1
12	8.766±6.74	5.666±2.8	7.748±2.8
24	7.064±4.95	4.903±3.4	5.963±2.1

Table 2: The pharmacokinetic profile of gliclazide before and after pre-treatment with ornidazole

Parameter	Gliclazide	Combination (9th day)
C_{max} (μg/ml)	2.26±0.16	4.471±0.51***
AUC_{total} (μg/ml*hr)	12.95±1.68	27.53±4.47***
$t_{1/2}$ (hr)	5.98±1.8	5.58±0.75^{NS}
MRT (min)	6.46±51.85	8.83±31.44^{NS}
Cl (ml/ hr)	391.65±3.03	185.61±0.86***
Vd (ml)	3250.8±873.1	1478.09±204.81**

Statistically significant at ***P<0.0001 and **P<0.0022 when compared with gliclazide control, NS- non significant. (Unpaired t test).

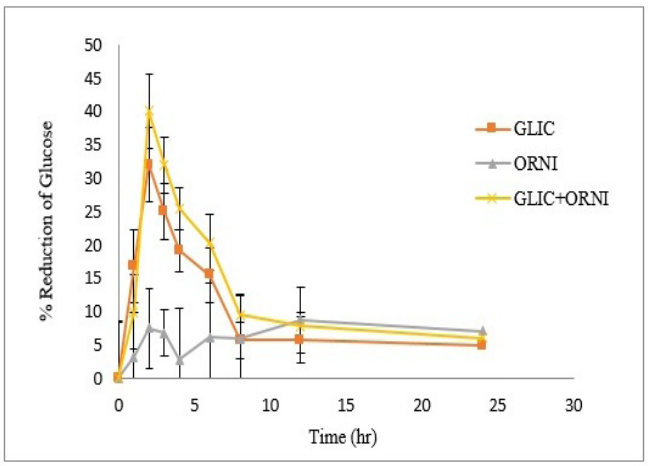

Figure 1: The comparison of mean ± SD of percentage reduction of blood glucose-time profile of gliclazide (2 mg/kg), ornidazole (25 mg/kg) and gliclazide + ornidazole combination

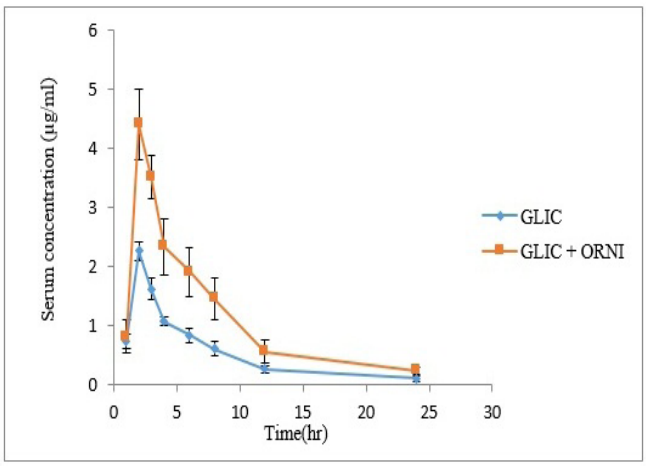

Figure 2: The comparison of mean ± SD of plasma concentration-time profile of gliclazide (2 mg/kg) before and after pre-treatment with ornidazole (25 mg/kg)

mg/kg), ornidazole (25 mg/kg) and gliclazide + ornidazole combination. As shown in the (Figure 2) there occurred significant changes in the plasma concentration time profile and pharmacokinetic parameters of gliclazide like C_{max}, AUC_{total}, C_l and V_d on multidose treatment with ornidazole as shown in (Table 2).

DISCUSSION

Drug interactions are usually seen in clinical practice and the mechanism of interactions are evaluated using animal models. Healthy rat model served to quickly identify the interaction.[11] In the present study we studied the influence of ornidazole on the pharmacodynamics and pharmacokinetics of gliclazide. Ornidazole enhanced the hypoglycaemic activity of gliclazide on multidose treatment (31.962 ± 5.4 to 40.032 ± 5.5) at second hour however it is found to be statistically non-significant.

Statistically significant alterations in pharmacokinetic parameters was observed, there is about 49.4%, 52.9%, 26.8% increase in C_{max}, AUC_{total} and MRT respectively. Clearance and V_d have decreased by 54.5% and 52.5% respectively. The potentiation of hypoglycemic effect of oral antidiabetic agents and increase in serum concentrations of gliclazide may be due to the inhibition of CYP2C9 isoenzyme system by pre-treatment of Ornidazole. The possible mechanism behind the interaction is CYP2C9 inhibition by ornidazole, the enzyme responsible for the metabolism of gliclazide.[12]

CONCLUSION

Our study confirmed that the interaction observed to be pharmacokinetic type and concludes ornidazole enhances plasma concentration of the gliclazide, which needs slight dose adjustment and care should be taken when the combination is prescribed for their clinical benefit in diabetic patients. However, the present study is limited to describe the exact mechanism of action(s) behind this interaction and it has to be confirmed by conducting pharmacokinetic interaction studies in different species. Whether or not similar Interaction will occur in humans is needed to be investigated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Highlights of Paper

- Pharmacokinetic interaction of gliclazide with ornidazole in healthy Albino Wistar rats were studied.
- Results demonstrated that the ornidazole alone does not possess glucose lowering activity.
- Drug-drug interaction of ornidazole with gliclazide is pharmacokinetic type and the possible interaction may be due to CYP2C9 inhibition.
- The combination of ornidazole and gliclazide may need slight dose adjustment and care should be taken when the combination is prescribed for their clinical benefit in diabetic patients

Author Profile

- Madhavi Meesa (Corresponding Author) currently working as an Associate professor in the department of pharmacology at Vaagdevi College of pharmacy Warangal. In this regard, I would like to inform you that my area of interest is to develop and validate sensitive bioanalytical methods by HPLC for several drugs using rat serum/plasma to study the pharmacokinetics and also to Analyze and assess pharmacokinetic drug interaction between different drugs in humans and rats.In additional, to understand drug metabolism and pharmacokinetics (DMPK), in particular role in drug discovery and development.Iam grateful to all my coauthors for their full support and contribution throughout the research work. Till now I have published more than two articles in different journals..

- Mamatha Mada (co-author) have done M. Pharmacy in the department of pharmacology at Vaagdevi College of pharmacy waranagal. Currently working as Executive-Medical Writer to Design and Development of Protocols and the informed consent forms for Bioavailability/Bioequivalence studies in Piramal Clinical Research a Division of Piramal Healthcare Ltd, Hyderabad. My area of interest is to analyze and assess pharmacokinetic drug interaction between the different drugs and also to understand drug metabolism and pharmacokinetics (DMPK), in particular role in drug discovery and development.

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