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Research Article

INFLUENCE OF FLUOXETINE ON PHARMACOKINETICS AND PHARMACODYNAMICS OF GLIBENCLAMIDE IN DIABETIC RATS

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ABSTRACT

Objectives: The aim of the present study was to evaluate the influence of Fluoxetine (antidepressant drug) on pharmacokinetic and pharmacodynamics of Glibenclamide (antidiabetic drug) in diabetic rats.

Methods: Alloxan-induced Diabetic model in rats has been used in this study. After induction of diabetes, Glibenclamide (10mg/kg/p.o or 5mg/kg/p.o) and Fluoxetine (5.4mg/kg/p.o) were administered orally for 7days. The Pharmacokinetic parameters like $t_{1/2}$, AUC, Clearance, T_{max} and C_{max} of Glibenclamide, with and without combination of Fluoxetine treatment were determined. The blood glucose levels were estimated using Glucose Oxidase-Peroxidase(GOD-POD) method.

Results: The increase in Glibenclamide concentration might be due to interaction with Fluoxetine at metabolic enzyme (CYP 3A4 ,CYP 2D6 and CYP 2C19 Isoenzymes). Increase in plasma concentration increases AUC of Gibenclamide indicates the raised bioavailability in presence of Fluoxetine.Glibenclamide treatment in combination with Fluoxetine has shown significant decrease in Blood Glucose Levels when compared to rats treated with Glibenclamide. The results of the present study indicated that Fluoxetine has influence on Pharmacokinetic and Pharmacodynamics of Glibenclamide. Thus concluding the concomitant administration of Glibenclamide and Fluoxetine should be used in caution. Further studies are to be done to reduce the dose of glibenclamide (antidiabetic drug) when it is given in combination with fluoxetine (antidepressant drug)

Keywords: Fluoxetine ,diabetes , depression .

INTRODUCTION

Pharmacokinetic drug–drug interactions (DDIs) are unfavorable clinical events, which are caused by abnormally increased or decreased drug concentrations in the body as a consequence of co-administration of other drug(s) (Sproule.*et.al.,*1997) and sometimes its metabolites at the effective sites within the body. The relationship between drug administration and response is divided in to two phases. Pharmacokinetic phase, which related to the body's effect on the drug and. Pharmacodynamic phase, which related to the drug effect on the body. Patients often receive multiple medications therapy simultaneously, in diseases such as Diabetes, Cancer and AIDS etc, which demand the combination therapy, which works better than an individual drug alone. In other cases, the patient is suffering from several conditions, each of which is being treated with one or more drugs, in this situation there is many potential sites for interaction that exist within the body. An interaction may occur between them by either altered Pharmacokinetics or Pharmacodynamics of one drug by another (Patsalos.*et.al.,*2003). Diabetes is certain to be among the most challenging health problems in the 21st century. According to prevalence estimates of the International Diabetes Federation, 366 million people have diabetes in 2011; by 2030 this will have risen to 552 million. An alternative hypothesis in which depression precedes and predisposes individuals to diabetes may explain the development of type 2diabetes among patients diagnosed with depression several years earlier (Han *et.al.,* 2002).The number of people with type II diabetes, which affects about 90% of all diabetic patients, is increasing in every country. Depression has become a global problem, and major depression is currently leading globally, in the cause of disability, and has become a serious public health problem (Ustun *et.al.,* 2004).Depression is a serious medical illness that involves the brain. Depression is a medical condition or illness involving various physiological, affective and cognitive manifestations. The high prevalence of co-morbid depression and diabetes suggests that these disorders may be related (Talbot *et. al.,* 2000) Depression has been proposed as both a result of and precursor to diabetes, with this relationship attributed to a variety of mechanisms. Studies have examined a number of psychosocial and biological correlates of depression and diabetes, yet the causal relationship between these disorders, its direction, and underlying mechanisms remain unclear (De-Groot M *et.al.,*2001). Contradictory research findings suggest the relationship between depression and diabetes is complex and may differ for type 1 and type II diabetes. The factors underlying this relationship may be bidirectional and consist of multiple mechanisms and/or indirect causation. Golden et al found evidence for a bidirectional relationship within the same cohort; adults with treated type II diabetes who were depression-free at baseline were 52% more likely to experience depressive symptoms than non-diabetics, while adults with elevated depression scores were 21% more likely than those with low or normal symptoms to develop type II diabetes after adjusting for clinical, demographic, and lifestyle risk factors (Baldwin *et.al.*, 1999). In the present study, we have evaluated the effect of Fluoxetine (antidepressant drug) on pharmacokinetic and pharmacodynamics of Glibenclamide (antidiabetic drug) in diabetic rats.

MATERIALS AND METHODS

Experimental animals: Adult male albino wister rats weighing 140-200g (4-8 weeks) were used for the study. They were housed in polypropylene cages and were maintained at room temperature of 23°C ± 2°C and relative humidity 50%. They were maintained in 12h:12hr light:dark cycle throughout the period of acclimatization and experimental study. Animals were provided with standard rodent pellet diet. Food and water was allowed *ad libitum.* All wistar rats were feed a normal laboratory chew diet (Nutrilab Rodent Fed, PROVIMI) containing (W/W) of 21.88% crude proteins, 52.15% carbohydrates and 5.97% crude fat. The experiments were planned after the approval of Institutional Animal Ethical Committee (IAEC).

Induction of Diabetes in rats

Diabetes mellitus or hyperglycemia was induced in overnight fasted rats by administration of alloxan monohydrate (2, 4, 5, 6,-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) at dose of 120 mg/kg intraperitoneally in normal saline (Ragavan and Krishnakumari, 2006). After one hour of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation for about 48 hours. The animals were kept fasting overnight and fasting blood glucose levels were estimated before and after 72 hrs of alloxan treatment. Animals showing blood glucose levels >200 mg/dl is considered as diabetic and were used for study.

Experimental Study Design: The diabetic rats were divided into following groups of each consists of 5 rats.

Group I : Normal control group treated with vehicle (0.5%Sod CMC)
Group II : Diabetic control group treated with vehicle (0.5%Sod CMC)
Group III : Diabetic rats treated with Glibenclamide (10mg/kg/p.o)
GroupIV : Diabetic rats treated with Glibenclamide(10mg/kg/p.o) and Fluoxetine(5.4mg/kg/p.o)
Group V : Diabetic rats treated with Glibenclamide (5mg/kg/p.o) and Fluoxetine (5.4mg/kg/p.o)

Treatment was continued for 7 days and on the first (single day study) & last day (multiple day study), blood samples were collected through retro orbital puncture method and centrifuged to separate plasma and stored at -20^{o}C.

Collection and analysis of blood samples

Blood samples were collected from all the group of rats in different intervals at 0min, 30min, 1hr, 2hr, 3.5hr, 6hr and 8hr after fasting for 12 hrs. The plasma samples were analysed for blood glucose levels (Trinder *et al.,* 1969) and also for plasma glibenclamide concentration to study the effect of fluoxetine on pharamcodynamics and pharmacokinetic of glibenclamide respectively.

Estimation of Glibenclamide in Plasma using HPLC

The plasma glibenclamide concentrations in treated rats were estimated using High Pressure Liquid Chromatography (Nanovskaya *et.al.,* 2006)

HPLC Conditions

Shimadzu SPD 10 UV Detector with Phenomenex (250×4.6mm) C18 5µm reverse phase analytical column was used. The mobile phase consisted of Buffer and Acetonitrile (30Mm:pH 5.8) in the ratios of 55:45 v/v. Before Use, the mobile phase was filtered by using it through a 0.45µm filter and the filtrate is degassed by using bath sonicator. The mobile phase was pumped at an isocratic flow of 1ml/min at room temperature. The peaks were determined using a detector set at a wavelength of 254nm with 3.98 min as retention time (RT). All the procedures were performed at ambient temperature.

Extraction procedure

The plasma samples were collected from all groups of rats. To the sample add 2ml of mobile phase and allow it for 15min sonication and centrifuged for 10 min at 3000 rpm. After centrifugation, the supernatant was transferred into a new tube,to the 2ml of supernatant liquid add 2ml of mobile phase from this 20 µl was injected into the HPLC.

Statistical Analysis

All the experimental Values are expressed as mean ± SD. The Blood Glucose levels were compared with diabetic control value at each time point using One-Way ANOVA followed by post Dunnet test. The statistical significance was judged at the 0.05 probability level. Statistical analysis were carried out using demo version of Graph Pad Prism.

RESULTS

Diabetes mellitus was successfully induced in the rats by the administration of alloxan monohydrate (120mg/kg body weight,i.p) and was confirmed by the elevation of blood glucose levels from 77.8 to 229.25 mg/dl.

Table 1: The blood glucose levels in rats treated with Glibenclamide alone and in presence of fluoxetine (Single Dose Study)

GROUPS	O hr	0.5 hr	1hr	2hr	3.5hr	6hr	8hr
Normal Group	77.8±0.45(0)	77.71±1.250 (+1.15)	78.7±2.56 (+5.26)	81.9±1.89 (+4.6)	81.4±3.61 (+5.3)	82.0±2.98 (+5.91)	82.4±3.1
Diabetic Control	229.25±6.79	230.50±6.59 (+0.54)	233.26±6.50 (+1.74)	238.00±6.22 (+3.81)	241.97±6.39 (+5.54)	245.01±6.99 (+6.87)	239.02±7.98 (+4.26)
Glibenclamide (10 mg/kg)	249.49±0.66	221.12±0.53 (-11.37) **	194.00±0.52 (-22.24) ***	165.05±0.49 (-33.84) **	142.52±1.1 (-42.87) **	128.12±0.52 (-48.64) **	148.08±5.5 (-40.64) **
Glibenclamide (10mg/kg) + Fluoxetine (5.4 mg/kg)	253.53±1.33	210.08±0.928 (-17.83) **	182.43±1.83 (-28.04) ***	156.12±0.98 (-38.42) ***	132.24±1.80 (-47.84) ***	112.56±1.40 (-55.60) ***	140.96±2.40 (-45.58) **
Glibenclamide (5mg/kg)+ Fluoxetine (5.4 mg/kg)	250.17±0.75	222.49±0.53 (-11.06) **	196.52±0.52 (-21.44) ***	167.77±0.49 (-32.93) ***	141.28±1.10 (-43.52) ***	130.52±0.52 (-47.82) ***	149.56±9.53 (-40.21) **

***All the* values are expressed as mean ± S.D (n=5) **p<0.01, ***p<0.001 vs diabetic control**
( ) indicates % reduction of Blood glucose level, -ve sign indicates reduction in Blood glucose level,+ve sign indicates increase in Blood glucose level.

Effect of fluoxetine on pharmacodynamics of glibenclamide

The blood samples from all the groups of rats on 1st and 7th day of treatment at different intervals and were analyzed for fasting glucose levels to assess the effect of fluoxetine on pharmacodynamics of glibenclamide in diabetic rats and the results were showed in tables 1-2. Treatment with glibenclamide at a dose of 10mg/kg reduced the blood glucose levels with time dependent

manner ($p < 0.01$) . The maximum reduction of blood glucose level were observed with glibenclamide (10mg/kg) at 6th hr and it was 48.64% , where as it was 55.60% in presence of fluoxetine (5.4mg/kg). But the % reduction of blood glucose levels with treatment of glibenclamide half dose (5mg/kg) in presence of fluoxetine (5.4mg/kg) was 47.82 at 6th hr (table 1). In multiple dose interaction study, treatment with glibenclamide at a dose of 10mg/kg reduced the maximum blood glucose levels at 6th hr was 53.17% , where as it was 59.44% in presence of fluoxetine (5.4mg/kg) and it was 51.13 % with glibenclamide half dose (5mg/kg) in presence of fluoxetine (5.4mg/kg) (table 2).

Table 2: The blood glucose levels in rats treated with Glibenclamide alone and in presence of fluoxetine (multiple Dose Study)

GROUPS	O hr	30min	1hr	2hr	3.5hr	6hr	8hr
Normal Group	82.5±0.76(0)	81.25±0.98 (+1.5)	83.12±1.25 (+0.7)	82.05±0.65 (+0.6)	80.12±2.15 (-2.8)	76.56±3.27 (-7.2)	74.98±5.27 (-9.1)
Diabetic Control	255.47±17.47(0)	256.19±17.49 (+0.27)	257.65±17.19 (+0.86)	259.78±8.18 (+1.68)	262.36±16.70 (+2.66)	265.19±16.03 (+3.79)	266.15±18.85 (+4.18)
Glibenclamide (10 mg/kg)	167.47±0.78	147.12±0.81 (-12.15) ***	131.47±1.08 (-21.44) ***	111.41±0.93 (-33.47) ***	91.68±0.61 (-45.25) ***	78.42±0.59 (-53.17) ***	98.87±5.21 (- 40.96) ***
Glibenclamide (10 mg/kg) + Fluoxetine (5.4 mg/kg)	166.60±0.96	141.27±0.97 (-14.89) ***	126.78±0.80 (-23.62) ***	94.12±0.49 (-43.50) ***	80.12±1.04 (-51.90) ***	67.56±0.53 (-59.44) ***	89.56±8.76 (- 46.24) ***
Glibenclamide (5mg/kg)+ Fluoxetine (5.4 mg/kg)	170.05±1.09	156.12±1.10 (-8.19) ***	141.62±0.52 (-16.71) ***	118.56±0.80 (-30.27) ***	99.29±0.63 (-41.61) ***	83.15±1.04 (-51.13) ***	104.87±8.98 (- 38.33) ***

All the values are expressed as mean ±s.d (n=6)p<0.01, ***p<0.001 vs diabetic control**
( ) indicastes % reduction of Blood glucose level , -ve sign indicates reduction in Blood glucose level , +ve sign indicates increase in Blood glucose level.

Table 3: The Pharmacokinetics of Glibenclamide in presence & absence of fluoxetine Single Dose Study

Parameters	Glibenclamide (10mg/kg)	Glibenclamide(10mg/kg)+ Fluoxetine(5.4mg/kg)	Glibenclamide(5mg/kg)+ Fluoxetine(5.4mg/kg)
Vol area (ml)	127.332	103.409	130.176
Vol area (ml/kg)	636.661	517.046	650.882
$t_{1/2}$(h)	9.77	8.85	6.90
CL (ml/hr)	9.029	8.089	13.061
CL (ml/hr/kg)	45.148	40.446	65.306
Tmax (h)	6	6	6
Cmax (µg/ml)	1.21	1.45	1.1
AUC 0-t (µg/ml*h)	7.34	8.87	6.34
AUC 0-α (µg-hr/ml)	22.14	24.72	15.31
AUMC (0- t)	63.65	75.66	55.84
AUMC (0- α)	390.89	405.10	217.00
MRT(hr)	17.64	16.38	14.17

Table 4: The Pharmacokinetics of Glibenclamide in presence & absence of fluoxetine multiple Dose Study

Pk parameters	Glibenclamide (10mg/kg)	Glibenclamide (10mg/kg) + Fluoxetine(5.4mg/kg)	Glibenclamide(5mg/kg)+ Fluoxetine(5.4mg/kg)
Vol area (ml)	96.07	82.30	121.18
Vol area (ml/kg)	480.35	411.52	605.93
$t_{1/2}$(h)	7.06	6.38	8.260
CL (ml/hr)	9.42	8.93	10.16
CL (ml/hr/kg)	47.10	44.66	50.83
Tmax (h)	6	6	6
Cmax (µg/ml)	1.46	1.64	1.23
AUC 0-t (µg-hr/ml)	8.99	10.22	7.27
AUC 0-α (µg-hr/ml)	21.23	22.38	19.67
AUMC (0- t)	74.39	82.60	63.59
AUMC (0- α)	297.09	291.96	310.51
MRT(hr)	13.99	13.03	15.78

Effect of fluoxetine on pharmacokinetics of glibenclamide

The effect of fluoxetine on pharmacokinetics of glibenclamide was studied in single and multiple day interaction. The blood samples from all the groups of rats on 1st and 7th day of treatment at different intervals and were analyzed for glibenclamide concentrations and the results of estimated pharmacokinetic parameters were showed in tables 3-4.

Single dose interaction study

The plasma Glibenclamide concentrations were significantly increased in presence of fluoxetine (5.4mg/kg) and were reduced with half dose of glibenclamide in presence of fluoxetine. But the plasma concentrations in rats treated with Glibenclamide (5mg/kg)

in presence of fluoxetine (5.4mg/kg) were comparatively less than full dose of Glibenclamide and were near to those of glibenclamide

(10mg/kg) alone treated rats. The pharmacokinetic parameters like $C_{max,}$ AUC were comparatively more in Glibenclamide (10mg/kg) and fluoxetine treated rats than glibenclamide alone group (table 3).

Multiple dose interaction study

The pharmacokinetic parameters like $C_{max,}$ AUC were comparatively more in Glibenclamide (10mg/kg) and fluoxetine treated rats than glibenclamide alone group (table 4). In diabetic rats after Multiple dose treatment of Glibenclamide (10mg/kg) in presence of fluoxetine (5.4mg/kg) the pharmacokinetic parameters AUC and AUMC were increased when compared to glibenclamide (10mg/kg) alone and Glibenclamide (5mg/kg) in presence of fluoxetine (5.4mg/kg). The pharmacokinetic parameters AUC and AUMC of Glibenclamide (5mg/kg) in presence of fluoxetine (5.4mg/kg) are less when compared to the pharmacokinetic parameters AUC and AUMC of Glibenclamide (10mg/kg) alone but the pharmacokinetic parameters of Glibenclamide (5mg/kg) in presence of fluoxetine (5.4mg/kg) are very near to that of Glibenclamide (10mg/kg) alone.

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder requiring lifetime treatment and anti-diabetic drugs sulfonylureas including glimepiride, glibenclamide and glipizide will control the blood glucose levels (Calcutt *et.al.,* 2009). Depression is a psychological disorder, which also requires treatment for a specified or prolonged period and can be treated with SSRI's fluoxetine, fluvoxamine. If a patient is suffering with both these disorders require simultaneous treatment for a specified time period. (De-Groot *et.al.,*2001). Drug interactions are usually seen in clinical practice and the mechanisms of interactionas are evaluated usually in animal models. The present study evaluates the influence of Fluoxetine on pharmacokinetic and pharmacodynamic interaction with Glibenclamide. The results observed suggest that co-administration of Fluoxetine with Glibenclamide enhances antidiabetic activity, probably due to the enhanced bioavailabilty of Glibenclamide. In this study serum glibenclamide levels were significantly ($p<0.001$) increased by administration with fluoxetine along with glibenclamide at the dose of 10mg/kg. There was significant rise in serum glibenclamide levels and pharmacokinetic parameters like AUC, AUMC of glibenclamide with single and multiple dose treatments.The increase in AUC indicates improved bioavailabilty of glibenclamide in presence of Fluoxetine.The increase in bioavailability of glibenclamide was more in multiple doses than single dose study.

Fluoxetine was metabolized by hepatic P450 CYP $2C_9$ isoenzyme and there is more possibility inhibition of metabolism of glibenclamide which is also metabolised by both CYP 2D6 and CYP 2C9. (Preissner S et.al.,2009)**.** Hence interaction at hepatic metabolism with reduced glibenclamide metabolism by fluoxetine leading to raised serum level is possible.Decreased absorption rate costant indicates delay in rate of absorption.Elimination rate constant is increased indicating increased elimination.Tmax is constant.

Fluoxetine combination treatment has shown influence on pharmacokinetic (metabolism and distribution)of glibenclamide .The combination treatment of glibenclamide and fluoxetine has shown significant difference in reducing blood glucose.So, hereby the present study indicates that fluoxetine has influence on glibenclamide pharmacokinetics and pharmacodynamics.

CONCLUSIONS

The present study results suggest that, fluoxetine enhance the hypoglycemic activity of glibenclamide by enhancing the bioavailability of glibenclamide, possibly by the inhibition of CYP2C9 moderately and the results are statistically significant.Concomitant administration of Glibenclamide with Fluoxetine has shown significant pharmakinetic and pharmacodynamic interactions.Positive pharamcodynamic interaction between glibenclamide and fluoxetine was established resulting in enhanced antidiabetic potential of glibenclamide in combination with fluoxetine as comparedto glibenclamide alone.On pharmacokinetic interaction with fluoxetine bioavailabilty of glibenclamide is raised showing greater effect than single drug.

It can be concluded from findings of the present study that glibenclamide-fluoxetine is effective and safe for treatment ,thus suggesting cautionary use in diabetic and depression condition.

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